Review of the Environmental Protection Agency's Draft IRIS Assessment of Tetrachloroethylene

Committee to Review EPA's Toxicological Assessment of Tetrachloroethylene

Board on Environmental Studies and Toxicology

Division on Earth and Life Studies

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Preface

In June 2008, the U.S. Environmental Protection Agency (EPA) released its draft *Toxicological Review of Tetrachloroethylene (Perchloroethylene) (CAS No. 127-18-4) in Support of Summary Information on the Integrated Risk Information System (IRIS)*. The assessment provided estimates of cancer and noncancer effects, which will be used to establish air and water quality standards to protect public health and set cleanup standards for hazardous-waste sites. EPA requested that the National Research Council review the scientific evidence on the adverse health effect of tetrachloroethylene and the agency's application of such data in quantifying human health risks. The review was sought to ensure that the draft IRIS assessment was consistent with current EPA guidance on conducting risk assessments and that it reflected sound scientific analysis and judgment.

In response to EPA's request, the National Research Council convened the Committee to Review EPA's Toxicological Assessment of Tetrachloroethylene, which prepared this report. The members of the committee were selected for their expertise in pharmacokinetics, liver toxicology, kidney toxicology, neurotoxicology, hematopoietic toxicology, reproductive toxicology, developmental toxicology, genotoxicity, carcinogenesis, epidemiology, physiologically based pharmacokinetic modeling, biostatistics, and risk assessment. Biographic information on the committee members is provided in Appendix A.

To help the committee in its review, public meetings were held in November 2008 and January and April 2009 to gather information from EPA, academic and industry researchers, state public-health departments, and the general public. The committee is grateful to those who gave presentations on research related to tetrachloroethylene or on topics relevant to the committee's task, including Judith Schreiber, Office of the New York State Attorney General; Philip Bushnell, EPA; Thomas Burke, Johns Hopkins Bloomberg School of Public Health; Andy Salmon, California Environmental Protection Agency; and Harvey Clewell III, Hamner Institutes for Health Sciences. The committee also thanks Peter Preuss, Kathryn Guyton, and Karen Hogan for providing background information and responding to questions throughout the study.

One committee member, Rolf Schulte-Hermann, disagreed with the committee's support of EPA's conclusion that the mode of action of tetrachloroethylene in inducing liver cancer in rodents is unknown. He judges that the induction of liver cancer in mice can be fully explained by a mode of action that involves the activation of the peroxisome proliferator-activated receptor-alpha. The basis of his judg-

ment and of his dissent from the committee's position is detailed in Appendix B, where it is followed by the committee's rebuttal.

This report and the dissenting statement have been reviewed in draft form by persons chosen for their diverse perspectives and technical expertise in accordance with procedures approved by the National Research Council's Report Review Committee. The purpose of the independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards of objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We thank the following for their review of this report: A. John Bailer, Miami University; Lucio Costa, University of Washington; Scott E. Bowen, Wayne State University; Wolfgang Dekant, University of Würzburg; Adnan Elfarra, University of Wisconsin; Jeffrey Fisher, University of Georgia; David H. Garabrant, University of Michigan; Bernard D. Goldstein, University of Pittsburgh; David G. Hoel, Medical University of South Carolina; Ronald Melnick, National Institute of Environmental Health Sciences; Dorothy Patton, Environmental Protection Agency (retired); David Richardson, University of North Carolina School of Public Health; and Lauren Zeise, California Environmental Protection Agency.

Although the reviewers listed above provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations, nor did they see the final draft of the report before its release. The review of the report was overseen by the review coordinator, David Eaton, University of Washington, and review monitor, Mark Cullen, Yale University. Appointed by the National Research Council, they were responsible for making certain that an independent examination of the report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of the report rests entirely with the author committee and the institution.

The committee is grateful for the assistance of National Research Council staff in preparing the report, in particular Susan Martel, who served as project director and contributed to the report. Other staff members who contributed are James Reisa, director of the Board on Environmental Studies and Toxicology; Keegan Sawyer, associate program officer; Norman Grossblatt, senior editor; Mirsada Karalic-Loncarevic, manager of the Technical Information Center; Radiah Rose, editorial projects manager; and Tamara Dawson, program associate.

Finally, I thank all the members of the committee for their time and efforts throughout the development of this report.

Sam Kacew, *Chair*
Committee to Review EPA's
Toxicological Assessment of
Tetrachloroethylene

Abbreviations

AUC	area under the curve
BMC	benchmark concentration
BMCL	benchmark concentration with its lower confidence limit
BMD	benchmark dose
BuChE	butyrylcholinesterase
CCI	color-confusion index
CFU	colony-forming unit
CHO	Chinese hamster ovary
CI	confidence interval
CNS	central nervous system
CYP	cytochrome P-450
DCA	dichloroacetic acid
DEHP	diethylhexylphthalate
EBV	Epstein Barr virus
8-OHdG	8-hydroxydeoxyguanosine
EPA	U.S. Environmental Protection Agency
FDA	Food and Drug Administration
FMO	flavin-containing monooxygenase
GJIC	gap junctional intercellular communication
GSH	glutathione
GST	glutathione *S*-transferase
HD	Hodgkin disease
IARC	International Agency for Research on Cancer
IRIS	Integrated Risk Information System
JEM	job-exposure matrix
JISA	Japan Industrial Safety Association
JTEM	job-task exposure matrix
LGLL	large granular lymphocytic leukemia
LOAEL	lowest observed-adverse-effect level
MCL	mononuclear-cell leukemia
MOA	mode of action
***N*-Ac-TCVCS**	*N*-acetyl-*S*-(1,2,2-trichlorovinyl)-L-cysteine

NCI	National Cancer Institute
NES	Neurobehavioral Evaluation System
NHL	non-Hodgkin lymphoma
NK	natural-killer
NOAEL	no-observed-adverse-effect level
NRC	National Research Council
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OR	odds ratio
PBPK	physiologically based pharmacokinetic modeling
PCO	palmitoyl-CoA oxidation
POD	point of departure
PPARα	peroxisome proliferator-activated receptor-alpha
RCC	renal-cell carcinoma
RfC	reference concentration
RfD	reference dose
RfV	reference value
SAB	Science Advisory Board
SCE	sister-chromatid exchange
SIR	standardized incidence ratio
SMR	standardized mortality ratio
TCA	trichloroacetic acid
TCVC	*S*-(1,2,2-trichlorovinyl)-L-cysteine
TCVCS	*S*-(1,2,2-trichlorovinyl)-L-cysteine sulfoxide
TCVG	*S*-(1,2,2-trichlorovinyl) glutathione
TWA	time-weighted average
VCS	visual-contrast sensitivity
VEP	visual evoked potential
WHO	World Health Organization

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Review of the Environmental Protection Agency's Draft IRIS Assessment of Tetrachloroethylene

Summary

Tetrachloroethylene is a volatile, chlorinated organic hydrocarbon that is widely used as a solvent in the dry-cleaning and textile-processing industries and as an agent for degreasing metal parts. It is an environmental contaminant that has been detected in the air, groundwater, surface waters, and soil. In June 2008, the U.S. Environmental Protection Agency (EPA) released its draft *Toxicological Review of Tetrachloroethylene (Perchloroethylene) (CAS No. 127-18-4) in Support of Summary Information on the Integrated Risk Information System (IRIS)*. The draft IRIS assessment provides quantitative estimates of cancer and noncancer effects of exposure to tetrachloreothylene, which will be used to establish air-quality and water-quality standards to protect public health and to set cleanup standards for hazardous-waste sites.

At the request of EPA, the National Research Council convened a committee to conduct an independent scientific review of the draft IRIS assessment of tetrachloroethylene from toxicologic, epidemiologic, and human clinical perspectives. The committee was asked to evaluate the adequacy of the EPA assessment, the data and methods used for deriving the noncancer values for inhalation and oral exposures and the oral and inhalation cancer unit risks posed by tetrachloroethylene; to evaluate whether the key studies underlying the draft IRIS assessment are of requisite quality, reliability, and relevance to support the derivation of the reference values and cancer risks; to evaluate whether the uncertainties in EPA's risk assessment were adequately described and, where possible, quantified; and to identify research that could reduce the uncertainty in the current understanding of human health effects associated with tetrachloroethylene exposure.

COMMITTEE'S ASSESSMENT

The committee appreciates the extensive work that EPA has invested in the development of its draft assessment of tetrachloroethylene. However, the committee has identified concerns about some of the approaches that EPA used to evaluate the data on tetrachloroethylene and subjects about which inadequate information or rationales are used to support its risk assessment—factors that

call into question the soundness and reliability of EPA's proposed reference values and cancer risk estimates for tetrachloroethylene. One of the overarching weaknesses of the draft assessment was a lack of critical analysis of the data on which EPA relied in evaluating methodologic strengths and weaknesses. That lack was particularly evident in the assessment of the epidemiologic data: study selection and conclusions appeared to be based heavily on results that showed positive associations, and other data and the strengths and weaknesses of the selected studies were not adequately taken into consideration. The committee observed similar problems in its review of EPA's evaluation of the genotoxicity evidence, in which preference appeared to be given to studies that reported positive results. Specifically, EPA did not analyze studies critically with respect to their methodologic strengths and weaknesses, nor did it organize its discussion clearly to provide an integrated consideration of the weight of evidence on the genotoxicity of tetrachloroethylene. Other mode of action evaluations were also hampered in this way.

In the sections below, the committee evaluates EPA's noncancer and cancer assessments of tetrachloroethylene. The committee's recommendations focus on improvements that should be made by EPA in producing its final assessment and on improvements that EPA should pursue in the future when tetrachloroethylene is due for another update.

Noncancer Assessment

For noncancer effects of tetrachloroethylene, EPA proposes to set an inhalation reference concentration (RfC) and oral reference dose (RfD). Those are estimates (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure and a daily oral exposure of the human population (including sensitive subgroups), respectively, that are likely to be without appreciable risk of deleterious effects during a lifetime. EPA's proposed RfC is 0.016 mg/m^3 (2 ppb), and its proposed RfD is 0.004 mg/kg per day. Those values are based on the neurobehavioral outcomes of visual dysfunction and cognitive deficits observed in epidemiologic studies. A 1995 study by Altmann et al., in which adverse neurotoxic effects (as measured by deficits in vigilance, reaction time, and visual memory) were observed in people who lived near dry-cleaning facilities, was selected as the basis of the derivation of the RfC and RfD. The committee was asked to evaluate the selection of neurobehavioral outcomes in support of the RfC and RfD, the key study used, approaches to route-to-route extrapolation, and the characterization of the uncertainties associated with the data.

Critical Noncancer End Point and Studies

The committee found that EPA adequately supported its selection of neurotoxicity as the critical effect on which to base the RfC and RfD. The draft IRIS

document illustrates that neurotoxic effects are the most sensitive effects of tetrachloroethylene and that reference values based on neurotoxic effects would be protective against other noncancer effects that occur at higher concentrations.

EPA provides descriptions of the relevant neurotoxicity studies, but its evaluation of the epidemiologic literature could be improved by providing a critical evaluation of the validity of study designs and evaluation of the methods used for data collection and analysis, which the committee judges to be most important in selecting key studies. EPA chose the 1995 study by Altmann et al. as the critical one for determining the RfC and RfD because it involved an environmental exposure and used a standardized computer-assisted testing battery. Those are reasonable bases for the choice, but they do not outweigh methodologic deficiencies that seriously compromised the results of the study. Most important, the referent group was not appropriate. The group had more education than the exposed group and appeared to have pre-existing differences in cognitive abilities, which could account for its better test results. Evidence of residual confounding by education can be seen in the variability in reported results. For example, there was no association between tetrachloroethylene and visual evoked potentials; this is important because changes in the visual system and abnormalities in visual evoked potentials have been associated with tetrachloroethylene and other related solvents, and they are essentially unrelated to education. Other limitations of the study included the lack of a rationale for initial selection of study subjects, inadequacy of exposure characterization, and lack of a dose-response relationship. Finally, even though the test battery was performed properly, some of the tests have not been well validated with regard to what they reveal about brain damage.

Thus, the committee disagrees with EPA's selection of the 1995 Altmann et al. study as the basis of its risk calculations. In reviewing the database, the committee gave greater weight to studies that had the strongest methods; it neither chose nor excluded studies on the basis of their results. The set of studies that the committee judged to be more appropriate for supporting the RfC and RfD include those of Altmann et al. (1990), Cavalleri et al. (1994), Gobba et al. (1998), Echeverria et al. (1995), and Boyes et al. (2009).

Derivation of Reference Values

EPA derived sample inhalation reference values by using results from several supporting neurotoxicity studies for comparison with its principal study by Altmann et al. The committee found that some uncertainty factors were applied inconsistently; specifically, the application of the uncertainty factor to account for subchronic exposures in epidemiologic studies should be justified better. In some cases, EPA did not use such a factor; in other cases, it applied a value of 10 with weak justification.

The committee derived candidate values by using the same studies as EPA and additional studies. The committee found that the reference values from the strongest studies were in the range of 6-50 ppb (or 0.04-0.34 mg/m^3). That range is higher than the RfC of 0.016 mg/m^3 derived by EPA and is further supported when considered in the context of the full database (see further discussion below).

EPA extrapolated the results of inhalation studies to derive the oral RfD for tetrachloroethylene. Physiologically based pharmacokinetic (PBPK) modeling was used to support the route-to-route extrapolation. The rationale behind that approach is sound and adequately explained by EPA, and the choice of dose metric (blood area-under-the-curve) was appropriate and adequately supported by the available evidence. However, the three models used by EPA were formulated and validated with data from inhalation exposures; none was validated against blood concentrations that result from oral exposure. EPA empirically assumed a value for the rate of oral absorption of tetrachloroethylene; this assumption is inferior to direct estimation. Other PBPK models that use direct estimation are available, and their use may help to reduce the uncertainty in the assumed values; or additional PBPK models could be developed (see recommendation below for a harmonized PBPK model).

Graphical Presentation

EPA provides graphical comparisons of reference values, values that could be derived from supporting studies. Reference values derived from neurotoxicity data are presented, as are values based on other noncancer effects to illustrate dose dependence of multiple forms of observed toxicity. Overall, the committee supports the approach of presenting the evidence in this visual format. However, the committee recommends some revisions to improve illustration of the uncertainties being represented and to expand the presentation to include the larger body of literature on a particular end point to show how the RfC compares with sample reference values derived from studies that are methodologically sound but not judged to be critical for the RfC. Consistency between the RfC and such studies would provide additional support.

Figure S-1 provides an example illustration developed by the committee. It shows that the majority of sample values is centrally clustered, but there is a wide spread at the lower and higher ends. The overall range of the 19 sample reference values is 0.03-333 ppb (0.0002-2.6 mg/m^3), but the range is reduced to about 6-50 ppb (0.04-0.34 mg/m^3) when consideration is restricted to the five strongest studies. The RfC of 0.016 mg/m^3 calculated by EPA on the basis of the 1995 Altmann et al. study falls below the range. The figure shows that sample reference values that could be derived from the full database of neurotoxicity studies provide some support for the range.

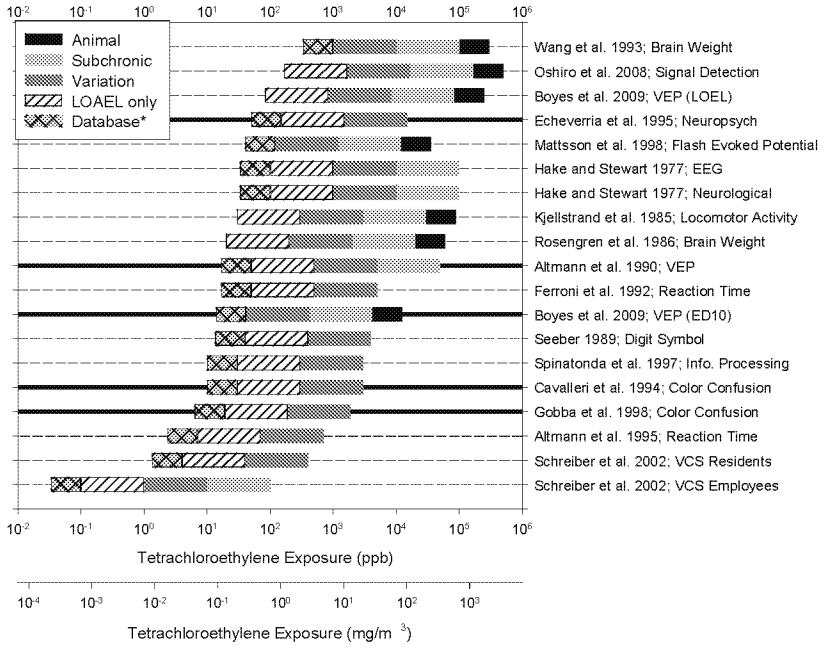

FIGURE S-1 Distribution of sample reference values. Each horizontal bar represents a single study. Thick, horizontal lines represent studies identified by the committee as most applicable to the development of an RfC. The right end of a bar is at the "point of departure" and is based on concentrations used in the referenced study after conversion to "human equivalencies" or, in the case of animal studies, after adjustment for continuous exposure. Uncertainty factors are illustrated in different shadings: a factor of 3 if it is necessary to extrapolate from animals to humans (black); a factor of 10 if it is necessary to extrapolate from acute or subchronic exposure to chronic exposure (light gray); a factor of 10 for individual variation to account for sensitive individuals (dark gray); a factor of 10 if the study did not contain a NOAEL (diagonal lines) and a factor of 3 for uncertainty in the data base as applied by EPA (light gray, cross-hatched). *A maximum total uncertainty factor of 3,000 was applied for the purpose of this exercise. Where this might be exceeded, the maximum was achieved by omitting the "database" uncertainty so that other uncertainties could be visualized. The committee has recommended that EPA review the uncertainty factors to ensure that they are appropriately explained and used consistently, so some of the individual values used here could be subject to change. In some cases, EPA might judge that the total uncertainty exceeds 3,000 and would, therefore, not use that study to derive a sample reference value. Source: Graphic developed by M. Christopher Newland.

Cancer Assessment

EPA faced a formidable challenge in its effort to characterize the carcinogenic properties of tetrachloroethylene both qualitatively and quantitatively.

There appears to be general agreement in the scientific community that tetrachloroethylene is carcinogenic in laboratory animals, but there is a longstanding debate about how to interpret and use the laboratory findings to predict human cancer risks. The debate is reflected in the committee's inability to reach consensus on some aspects of the tetrachloroethylene assessment, which are discussed below.

Classification

EPA classified tetrachloroethylene as "likely to be carcinogenic to humans." The committee reviewed the classification guidance in EPA's 2005 *Guidelines for Carcinogen Risk Assessment* and the bioassay data available on tetrachloroethylene and concluded that EPA adequately documented that its classification has been based on the results of bioassays that found increased incidences of hepatocelluar tumors, mononuclear-cell leukemia (MCL), renal tumors, and hemangiosarcomas in laboratory animals and to a lesser extent on epidemiologic evidence. EPA's decision to characterize tetrachloroethylene as likely to be a human carcinogen as opposed to "carcinogenic to humans" appropriately reflects the possibility that there are deficiencies or potential inaccuracies in interpretation of the data. Some of the possible deficiencies and inaccuracies are discussed below for each of the datasets.

Mononuclear-Cell Leukemia

An increased incidence of MCL in F344 rats has been reported in two bioassays. The biologic significance of the increases was debated by the committee because increases were observed in only one strain of rat, which is known to have a high background incidence of MCL, and because MCL's relevance to humans and the mode of action of tetrachloroethylene causing it are not understood. In considering the high background of MCL, the committee found a published assessment by Thomas et al. (2007) that applied statistical approaches (life-table analyses) to bioassays of the National Toxicology Program (NTP) to interpret dose response relationships. Tetrachloroethylene was one of five chemicals of 500 tested by NTP that showed statistically significant increases in MCL in both male and female rats despite the high background rates. The publication advocated that such statistical evidence be supported with a weight-of-evidence analysis of biologic data before conclusions were drawn.

The committee found some support from epidemiologic studies that suggested an association between tetrachloroethylene and lymphoma, but the data were relatively weak and inconsistent. A difficulty in interpreting the findings is a difference of opinion about the human relevance of MCL. Some committee members judged that similarities between a form of human leukemia (natural killer-cell large granular lymphocyte leukemia) and rat MCL and results of mechanistic studies that the committee recommended be added to EPA's as-

sessment were adequate to establish human relevance; others believed that more research was needed to establish the relevance. The committee agreed that there was little information on a mode of action of tetrachloroethylene in increasing MCL and that it therefore was not possible to determine whether exposure to tetrachloroethylene results in initiation of new tumors or enhances the expansion or promotion of existing tumors.

Hepatic Cancer

Statistically significant increases in hepatic tumors were observed in male and female mice after oral or inhalation exposure. As in the case of MCL, the biologic significance of the increases was debated by the committee because $B6C3F_1$ mice have a high background incidence of hepatic cancer. However, the findings were reproduced in several studies conducted in different laboratories and showed a dose-response relationship. There is also fairly substantial information for characterizing potential modes of action of hepatic-tumor formation relative to the data available on MCL and renal cancer. Although the committee recommended that EPA revise its presentation of the mode-of-action evidence on tetrachloroethylene-related hepatic cancer to clarify its position, most of the members agreed with EPA that the mode of action is complex and remains to be established. The latter members also agreed that there was insufficient evidence to rule out human relevance. One member objected to those conclusions and to the committee's support of using hepatic cancer to quantify risk. He argued that in the absence of evidence of other contributing modes of action, the evidence is sufficient to conclude that the mode of action in mice is predominantly through activation of the peroxisome proliferator-activated receptor-alpha, a mode of action that he considered to be of little relevance to humans. His arguments are presented in a dissenting statement in Appendix B of the report.

Renal Cancer

Tetrachloroethylene caused a low rate of induction of renal tumors in rats. Although the increases were not statistically significant when compared with concurrent controls, EPA has used historical controls to calculate the chances of two of these rare carcinomas to occur by chance to be less than 0.001. Furthermore, a dose-response trend was shown against the low background and the tumors in the treated rats were malignant whereas the tumors in the controls were not. EPA provided a strong evaluation of the potential modes of action for tetrachloroethylene-induced kidney cancer. The committee agrees with EPA that the mode of action of tetrachloroethylene tumorigenesis is not understood but that a mutagenic mode of action cannot be ruled out. Thus, renal tumors observed in tetrachloroethylene-treated rats were considered relevant to humans although additional characterization of quantitative relevance is desirable.

Selection of Tumor Type for Quantitative Assessment

The committee was unable to reach consensus on the selection of the critical cancer end point. The majority of the members judged that the uncertainties associated with MCL (particularly the high background incidence, uncertainty about the dose-response relationship, and poor understanding of mode of action) were too great to support using MCL data rather than data on hepatic or renal cancer for determining quantitative estimates of risk. Those members judged that the use of the MCL data could be justified only if it is EPA's policy to choose the most conservative unit risk when considering options but that such justification should be distinguished as a policy decision, not a scientific one. They believed that a more scientifically defensible approach would be to use the dataset that has the least uncertainty rather than the dataset that yields the highest estimate of risk. In their judgment, the hepatic-cancer data would have the least uncertainty, followed by the data on renal cancer and MCL.

Other members judged that the MCL data should be used for cancer-risk estimation. Their opinions were based on the observation that reproducible, statistically significant increases in MCL in male and female rats above the background incidence of MCL were found and that MCL was the cancer end point with the highest magnitude of response. They believed that use of the most sensitive response to quantify cancer risk decreases the uncertainty associated with potential differences in metabolism and susceptibility to tetrachloroethylene among exposed populations. They concluded that additional statistical analyses of the dose-response data and the addition of supporting mechanistic information identified by the committee would strengthen the existing support of the use of MCL in the draft assessment.

Mode-of-Action Considerations

The modes of action[1] by which tetrachloroethylene produces increases in

[1]There was some disagreement among the committee members on what constitutes "modes of action" and "key events." In Section 4.4.4 of the draft IRIS assessment, EPA discusses several "topics" relevant to the mode of action for hepatic toxicity, including metabolism, receptor activation, genotoxic effects, and nongenotoxic effects. EPA's presentation treats those topics as separate modes of action, but metabolism is presented as a key event or a component of multiple modes of action. Some committee members judged that that treatment was appropriate as an introduction to a discussion of multiple modes of action and was consistent with EPA guidelines. Other members judged that although early key events may occur in different pathways, they converge to produce one effect; thus, these members hold the view that there is one mode of action for an observed effect for which there are a number of specified key events (early key events may be derived from a series of pathways). Despite those differing viewpoints, all members of the committee agreed that more focused analyses of the available evidence are necessary to support hypothesized modes of action.

MCL, hepatic cancer, and renal cancer were an important consideration in EPA's and the committee's evaluations of the evidence. The analytic framework described in EPA's cancer guidelines for considering hypothesized modes of action was best applied in the draft IRIS assessment's consideration of renal cancer. The evaluation focused on synthesizing the evidence to support the idea that multiple modes of action may play a role. However, for hepatic cancer, the committee found that the assessment lacked the organization to present and provide appropriate context for the evidence clearly. It therefore recommended that EPA revise its mode-of-action assessment for hepatic cancer to support better the conclusions that were drawn. Specifically, the committee suggested that the mode-of-action analyses would be improved by outlining the proposed sequence of hypothesized tetrachloroethylene-associated key events (possibly with a diagram). Transparency would be improved by presenting the details of experimental results in tabular form to allow the reader to understand more easily the relative potency of tetrachloroethylene, or its metabolites, in inducing both key events and tumors. In this context, species and strain differences could also be considered more easily. The goals of the presentation should be to lay out the timeline of key events explicitly in the context of dose, to evaluate concordance between early and late events, and to consider the relative contribution of chemical-specific data compared with information on categories of chemicals. This approach should be applied to each hypothesized mode of action. Even if the data are ultimately judged to be insufficient to support a hypothesis, the exercise can be used to identify critical data gaps and to inform the direction of future research.

Low-Dose Extrapolation

EPA's dose-response analyses of the various cancer datasets involved using several models to extrapolate to doses below the experimental range. EPA considered six datasets: hepatocellular adenoma or carcinoma in male and female mice, hemangiosarcoma in male mice, MCL in male and female rats, and renal tumors in male rats. It used the multistage model for each dataset because mode-of-action information was lacking or uncertain and the model was able to fit a broad array of dose-response patterns. However, because the studies used small numbers of dose groups and because the benchmark-dose software automatically fixed some parameters to zero to obtain convergence in model-fitting, the fitted models were nearly linear in the low-dose range. The imposed linearity explains the similarity among the slopes of the models and among the unit risks derived from the models. In the case of hepatocellular adenoma and carcinoma in male mice and MCL in female rats, EPA considered the fitted models acceptable solely on the grounds that statistical tests for goodness of fit had nonsignificant results ($p > 0.10$). The committee considers this to be a weak rationale in that the statistical significance of goodness-of-fit tests may not detect a poor fit when the number of animals per dose group is small. The questionable fitting of

the multistage model to some candidate datasets and insufficient consideration of alternative models contribute to underestimation of the overall uncertainties.

EPA adopted linear low-dose extrapolation, the default option, with several justifications. First, nonlinear, mechanistic models are unavailable for dose-response modeling because mode-of-action information on tetrachloroethylene is insufficient and support for dynamic models is unavailable. Second, because mathematical models are subject to uncertainties for low-dose extrapolation beyond the experimental dose range, linear extrapolation is more conservative than all sublinear (curvilinear) models. When individual thresholds in the human population are plausible, wide variation in threshold values typically implies a curvilinear shape of the dose-response relationship. Thus, linear extrapolation protects susceptible subpopulations. Third, a few of the candidate data, especially EPA's preferred male-rat MCL data, exhibit a linear dose-response relationship. Whereas those arguments are consistent with EPA's *Guidelines for Carcinogen Risk Assessment*, there is evidence in the candidate datasets that the underlying dose-response relationship can be supralinear (for example, in MCL in female rats). When that is the case, low-dose linear extrapolation is not conservative. EPA does not present the full ranges of variation and uncertainty in relation to model choice, in large part because it applied only linear or nearly linear dose-response models to all candidate datasets.

Age-Adjustment Factor

EPA did not apply an age-adjustment factor to its cancer risk assessment, because there is little evidence that tetrachloroethylene or its oxidative metabolites directly damage DNA, because information about genotoxicity of glutathione (GSH) metabolites in cell assays other than *Salmonella* or in vitro experiments is lacking, and because the mode of action of tetrachloroethylene has not been established. In addition, there are no data on differential sensitivity to tetrachloroethylene carcinogenicity among life stages. The committee agrees that those are adequate reasons for not using an age-adjustment factor but suggests that the rationale can be strengthened if EPA follows the committee's suggestions for improving its analysis of the genotoxicity data and mode-of-action evidence.

Physiologically Based Pharmacokinetic Models

Tetrachloroethylene can be viewed as being metabolized by three pathways. The predominant pathway is the cytochrome P-450 (CYP) pathway that produces metabolites that have been associated with hepatic cancer. Two other pathways involve the GSH conjugation pathway that produces metabolites that are further metabolized by the β-lyase pathway or the β-lyase-independent pathway, each of which produce metabolites that have been associated with renal cancer. To take those metabolic factors into account, EPA used three PBPK

models to estimate human equivalent doses from animal studies and to perform route-to-route extrapolations. Each of the models used total metabolism of tetrachloroethylene as the dose metric. In some instances, EPA used a single model; in others, it used all three. The justification for using single or multiple models is not always clear. The committee observed that the models could yield different results because they were calibrated with different datasets, so comparisons among them were not straightforward. For consistency and to allow for better comparisons among end points, the committee recommends that EPA use a single PBPK model for its assessment. Ideally, the model would be a "harmonized" version of the three models used by EPA or of other relevant models (that is, a single model that integrates multiple exposure routes and tissue compartments).

The committee notes that the use of total metabolism as the dose metric for carcinogenicity reflects primarily the CYP metabolic pathway because of large differences in the flux of the metabolism between it and the GSH pathway. Using that dose metric does not reflect the contribution of the GSH conjugation pathway, which has been implicated in the development of renal cancer. EPA did not pursue the addition of the GSH pathway to any of the PBPK models, arguing that data on GSH-dependent metabolism are from in vitro studies or constitute measurements of urinary excretion products and do not represent toxic species in vivo. The committee agrees that the available data on the GSH pathway are more limited than the available data on the CYP pathway but notes that in vitro and urinary metabolite data were used in the development of the CYP-based PBPK models chosen by EPA. Thus, better justification is necessary to rule out modeling the GSH pathway.

The committee recommends that EPA explore the possibility of adding the GSH pathway to a harmonized PBPK model. If such modeling is determined to be infeasible, total metabolism can be used as a reasonably conservative dose metric. The modeling exercise would be useful in identifying data gaps that prevent successful modeling, which can be used to guide research that will allow more comprehensive PBPK models to be developed in support of the next IRIS reassessment of tetrachloroethylene.

Uncertainty Analysis

EPA has clearly identified key sources of uncertainty as part of its process of assessing the cancer risk posed by exposure to tetrachloroethylene, including human population variation, low-dose extrapolation, dose metrics, extrapolation from animals to humans, and the use of PBPK models for route-to-route extrapolation. The effect of uncertainties on risk estimates is assessed qualitatively in most parts of the IRIS draft except in dealing with such issues as the choice of dose-response models, the use of PBPK models, and, to a small degree, variation between studies. That approach reflects the current state of practice of uncertainty analysis.

In a few respects, the committee disagrees with EPA's presentation on uncertainties. For example, EPA notes narrow variation between cancer risks derived from four dose-response models. However, in its comparison, EPA used only data on male rats, and all four models were linear or nearly linear at lower doses. Failure to consider a wider array of feasible dose-response models, including multistage models of various orders, could lead to inadequate quantification of uncertainty associated with the choice of dose-response model.

The committee supports EPA's quantitative assessments of uncertainty with regard to choice of dose-response models, the use of PBPK models, and variation between studies. In particular, the committee found EPA's consideration of uncertainty due to different forms of dose-response models to be valuable, and it recommends that such quantitative evaluations be extended to all candidate datasets so that a fuller array of uncertainties can be assessed.

CONSIDERATIONS FOR FUTURE RE-EVALUATIONS OF TETRACHLOROETHYLENE

The committee found several parts of the draft IRIS assessment that could be improved on in the future. Such changes are not necessary for completing the current assessment but should be considered when tetrachloroethylene is re-evaluated in the future. They include improving transparency in selection and analysis of data, particularly with regard to uncertainty analysis. The committee encourages EPA to consider the most recent guidance from the National Research Council report *Science and Decisions*.

Organization and Approach

There is a vast amount of literature on tetrachloroethylene, and the draft IRIS assessment was hampered by having to manage the sheer volume of information on the chemical. Any new reassessment should begin with problem formulation and issue identification, consideration of whether to rely on previous reviews, determination of the focus of the new effort, and identification of the specific issues on which the reassessment is likely to focus. That would help to identify where multidisciplinary input at early stages of reanalysis should be sought, such as in data selection and mode-of-action evaluations in the context of risk-assessment practices. The process would include a delineation of criteria for selecting studies, approaches for conducting a weight-of-evidence evaluation, and options for dose-response assessment and the characterization of uncertainties. EPA should also consider ways to reorganize the document to streamline presentation of the data and analyses.

Uncertainty Analysis

EPA's assessment of tetrachloroethylene follows a traditional approach to developing cancer slope factors and hazard indexes that takes uncertainties into account qualitatively and via uncertainty factors. EPA states that it has introduced a new method for uncertainty analysis in the context of the dose-response assessments for tetrachloroethylene, but the only notable differences between its tetrachloroethylene assessment and those of other chemicals are the consideration of multiple end points and the limited use of bootstrap simulation for only a portion of uncertainties. EPA's uncertainty analysis remained typically focused on individual sources of uncertainty, and the analysis was often qualitative without presenting a full range of the uncertainty. Without an in-depth illustration of the propagation and cumulative effect of the uncertainties on the final risk estimate, quantification of the overarching uncertainty surrounding the final risk assessment is not possible. The committee notes that the current state of practice in quantitative uncertainty analysis does not fully meet the spirit of principles, guidelines, and recommendations that have accrued in recent years.

1

Introduction

Tetrachloroethylene is a volatile chlorinated organic hydrocarbon that is widely used as a solvent in the dry-cleaning and textile-processing industries and as an agent for degreasing metal parts. It is also used as a chemical precursor for synthesis of fluorocarbons. It has the following use pattern: 55% as a chemical intermediate, 25% for metal-cleaning and degreasing, 15% for dry-cleaning and textile-processing, and 5% for other unspecified uses (ATSDR 1997; EPA 2008). Dry-cleaning facilities are an important source of atmospheric emissions of tetrachloroethylene. Tetrachloroethylene becomes a groundwater contaminant as a result of leaks and improper disposal practices; it can persist in groundwater for years because it has little contact with air. The U.S. Environmental Protection Agency (EPA) has classified tetrachloroethylene as a hazardous air pollutant under the Clean Air Act, a toxic pollutant under the Clean Water Act, a contaminant under the Safe Drinking Water Act, a hazardous waste under the Resource Conservation and Recovery Act, and a hazardous substance under the Comprehensive Environmental Response, Compensation, and Liability Act.

EPA's Integrated Risk Information System (IRIS) is a database that provides the agency's assessments of potential human health effects of exposure to various substances in the environment. IRIS assessments provide quantitative estimates of cancer and noncancer effects that are used to establish air and water quality standards to protect public health and set cleanup standards for hazardous-waste sites. For noncancer effects, EPA establishes an oral reference dose (RfD) and an inhalation reference concentration (RfC), which are estimates (with uncertainty spanning perhaps an order of magnitude) of daily oral exposure and continuous inhalation exposure of the human population (including sensitive subgroups), respectively, that are likely to be without an appreciable risk of deleterious effects during a lifetime. For cancer, the IRIS database provides a characterization of the weight of evidence of human carcinogenicity, oral slope factors, and inhalation unit risks. An oral slope factor is an upper bound, approximating a 95% confidence limit, on the increased cancer risk posed by

lifetime exposure to an agent; it is usually expressed in units of proportion (of a population) affected per milligram per kilogram of body weight per day. A unit risk is the upper bound on the excess lifetime cancer risk estimated to result from continuous exposure to an agent at a concentration of 1 µg/L in water or 1 µg/m^3 in air. For example, a unit risk of 2×10^{-6} per microgram per liter is interpreted as 2 excess cancer cases (upper-bound estimate) expected to develop per 1,000,000 people if they are exposed to the chemical daily for a lifetime at 1 µg per liter of drinking water.

EPA requested that the National Research Council undertake an independent assessment of its draft *Toxicological Review of Tetrachloroethylene (Perchloroethylene) (CAS No. 127-18-4) in Support of Summary Information on the Integrated Risk Information System (IRIS),* hereafter called the draft IRIS assessment. The draft IRIS assessment proposes an RfC of 1.6×10^{-2} mg/m^3, an RfD of 4×10^{-3} mg/kg-day, a range of inhalation unit risks of 2×10^{-6} to 2×10^{-2} per mg/m^3, and a range of oral slope factors of 1×10^{-2} to 1×10^{-1} per mg/kg-day. EPA requested a review of those values and their scientific basis in 2006 but delayed public release of the draft IRIS assessment for additional evaluation within the agency. Therefore, the committee's review did not begin until June 2008, when the draft was released.

STATEMENT OF TASK

A committee convened by the National Research Council was asked to conduct a scientific review—from toxicologic, epidemiologic, and human clinical perspectives—of EPA's draft IRIS assessment of tetrachloroethylene that was made available for external review. The committee's review was to include an evaluation of the adequacy of the assessment and the data and methods used for deriving the RfD and RfC of tetrachloroethylene and its oral and inhalation cancer unit risks. The committee was asked to evaluate whether the key studies underlying the draft IRIS assessment were of requisite quality, reliability, and relevance to support the derivation of the RfD, RfC, and oral and inhalation unit risks; to evaluate whether the scientific uncertainties in EPA's risk assessment were adequately described and, where possible, quantified; and to identify research that could reduce the uncertainties given the current understanding of human health effects associated with tetrachloroethylene exposure.

During the study course of the project, EPA submitted specific questions for the committee to address. The final list, submitted in February 2009, included the following questions:

General Charge Questions:

1. Does the draft IRIS assessment provide a scientifically sound, balanced, and transparent review and synthesis of the key scientific evidence on chronic noncancer and cancer hazard and risk?

2. Please identify any additional important studies that should be considered in the assessment of the chronic noncancer and cancer health effects of tetrachloroethylene.

Specific Charge Questions:

Noncancer Assessment

1. Selection of neurotoxicity as the basis for the RfC and RfD for tetrachloroethylene—a number of studies assessing neurobehavioral and other effects in both humans and rodents are available for RfC and RfD analysis.
   a. Is EPA's selection of neurotoxicity, specifically visual dysfunction and cognitive deficits, appropriate for providing a point of departure for derivation of the RfC and RfD? The goal of a reference value is to provide an estimate of exposure of the human population (including susceptible subgroups) that is likely to be without appreciable risk of adverse health effects over a lifetime.
   b. Does EPA provide a sound and transparent description of the relevant studies of the neurotoxic effects of tetrachloroethylene?
   c. Does the assessment present an appropriate rationale for selection of the study by Altmann et al. (1995) as the critical study? If another study is judged more appropriate for use as the critical study, please provide a critical evaluation of it and of its suitability for meeting the goals of a reference value.

2. Characterization of Uncertainties—the noncancer assessment considers uncertainty on the basis of extrapolation from laboratory animals to humans, variations in response within experimental species, human variation, and database deficiencies; the noncancer RfC and RfD are based on a specific neurotoxicity effect; EPA also presents reference values based on other effects to illustrate the dose dependence of the multiple observed toxicities.
   a. Has EPA accurately and clearly characterized the basis of selection of uncertainty factors for the RfC and RfD? Please comment on the rationales underlying the choice of uncertainty factors, such as the database uncertainty factor, which is intended to account for the degree of limitations in both human and animal data.
   b. Please comment on EPA's graphic presentation of noncancer reference values that could have been derived from studies of different neurotoxic effects or toxic effects in other organ systems.

Cancer Assessment

1. Weight-of-evidence descriptor—the assessment concludes that tetrachloroethylene is "likely to be carcinogenic to humans" by all routes of exposure

within the framework of the *Guidelines for Carcinogen Risk Assessment* (EPA 2005a).

a. Does EPA provide a clear and cogent weight-of-evidence evaluation?
b. Does the assessment support the conclusion that tetrachloroethylene by oral and inhalation exposure is likely to be carcinogenic in humans (at all levels of exposure)?

2. Mode of action considerations—the mode of action of a carcinogen can inform identification of hazards and approaches used for a dose-response relationship; the assessment concludes that a mode of action of tetrachloroethylene has not been definitively established for any of the site-specific tumor types.

a. Does EPA provide a sound evaluation and characterization of the available data related to mode(s) of action for the carcinogenicity of tetrachloroethylene?
b. Do the available data support EPA's conclusion that mode(s) of action for tetrachloroethylene-induced carcinogenesis is unknown?
c. Does EPA clearly address why age-dependent adjustment factors for cancer risk are not applied, according to the *Guidelines for Carcinogen Risk Assessment* (EPA 2005a) and *Supplemental Guidance for Assessing Cancer Susceptibility from Early-Life Exposure to Carcinogens* (EPA 2005b)?

3. Development of the inhalation unit risk and oral slope factor—EPA's draft unit-risk estimate relies on choices of tumor type, point of departure, and low-dose extrapolation that aim to provide a "reasonable upper bound estimate" of risk; because the draft assessment judged that there was no strong basis for preferring one physiologically-based pharmacokinetic model over another, a range of tetrachloroethylene unit-risk estimates calculated with three PBPK models is given.

a. Please comment on EPA's selection of mononuclear-cell leukemia in male rats from the Japanese Industrial Safety Association study for quantitative derivation of the inhalation unit risk and oral slope factor. Note that, consistently with the *Guidelines for Carcinogen Risk Assessment* (EPA 2005a), the draft IRIS assessment does not infer site concordance of tumors across species. If another study or end point is judged to be more appropriate for the derivation of these risk values, please provide a critical evaluation of the end point and its suitability for supporting a unit risk estimate.
b. Does EPA clearly and objectively describe the low-dose extrapolation approach, that is, linear extrapolation in accordance with default recommendations in the *Guidelines for Carcinogen Risk Assessment* (EPA 2005a)?

4. Consideration of uncertainties—the cancer assessment considered the contribution of a number of sources of uncertainty; some uncertainties (for example, pertaining to mode of action and human sensitivity and variability) were qualitatively expressed, and in other cases EPA examined the potential quantita-

tive impact on the risk estimate; in addition to the unit risk estimate, the assessment provides lower bounds (such as confidence limits) and central estimates.

a. Has EPA identified and described the key sources of uncertainty in assessing cancer risks posed by tetrachloroethylene?
b. Is this analysis transparent and presented at a suitable level of detail for the IRIS assessment?
c. Does the assessment clearly and objectively present the choices made in developing reasonable upper-bound estimates of cancer risk posed by tetrachloroethylene?
d. The assessment includes tabular presentations of point-of-departure-based analyses that use different end points and approaches (see Tables 6-2, 6-3, 6-4, and 6-5). Is the information clearly presented and appropriately characterized?
e. In Section 6.2.2.2, the assessment presents exploratory calculations of potential probabilities of tumor response at low dose by using different functional forms. Is this analysis clearly presented and appropriately characterized?
f. Please discuss research subjects likely to characterize uncertainties better in future tetrachloroethylene cancer risk assessments.

Choice of Dose Metrics for Various Toxic Outcomes, PBPK Modeling, and Interspecies Scaling Approaches

Exposure to tetrachloroethylene results in the production of several metabolic products. The parent compound is used as the dose metric for neurotoxic effects, and the rate of formation of total metabolites in humans is used for cancer effects. Metabolite formation was modeled by using three PBPK models, which led to a range of cancer risk factors.

1. Please comment on the PBPK application for route-to-route extrapolation in developing an RfD and an oral slope factor from studies of inhalation exposure.
2. Please comment on the sufficiency of the available data to identify whether the parent compound or specific metabolites are responsible for the induction of cancer through tetrachloroethylene exposure.
3. Has EPA clearly and objectively presented
   a. Choice of dose metrics for different outcomes and their use in PBPK models?
   b. Strengths and weaknesses of different modeling approaches?
   c. The approach used in deriving the toxicologically equivalent human dose, including the application of an interspecies scaling factor ($BW^{3/4}$) to the fraction of the administered rodent dose that is metabolized?

4. Is EPA's conclusion that there is not a strong basis for preferring any one PBPK model for use in the risk assessment soundly and transparently characterized?

COMMITTEE'S APPROACH

The committee reviewed the material presented in EPA's draft IRIS assessment for scientific soundness, balance, and transparency. By the nature of the charge, the focus was on parts of the document that were critical for determining neurotoxicity and cancer end points. The review included evaluation of some of the primary literature cited by EPA, its approaches to evaluating and modeling data, and options for performing qualitative and quantitative assessment of uncertainties. Public comments submitted to EPA and to the committee on the draft assessment were considered. The committee also held public meetings at which it had the opportunity to ask questions of EPA staff, to obtain input from invited speakers who were doing research on tetrachloroethylene or related scientific issues, and to hear from other interested parties.

To identify new studies that should be considered in EPA's IRIS assessment, the committee performed a literature search for papers published from July 2004 (the official cutoff for EPA's comprehensive literature search) to March 2009. For the purposes of its review, the committee restricted its searches to MEDLINE and EMBASE. MEDLINE is produced by the U.S. National Library of Medicine and covers over 5,200 biomedical journals published in the United States and over 80 foreign countries. EMBASE is produced by Elsevier Science and indexes over 4,800 journals with a focus on the international literature. A simple search for "tetrachloroethylene," its synonyms, and its Chemical Abstracts Service registry number was performed. Literature retrieval was limited to studies pertinent to the evaluation of adverse health effects, such as toxicology studies (including studies on toxicokinetics and mode of action) and epidemiology studies.

Other sources of information that the committee considered included compilations of toxicology and human health information from national and international agencies and organizations, such as the Agency for Toxic Substances and Disease Registry, the International Agency for Research on Cancer, the California Environmental Protection Agency, and the European Union. Relevant publications from the National Research Council and the Institute of Medicine were also consulted. The committee and staff examined the reference lists included in EPA's draft assessment, major epidemiologic studies, review articles, and major compilations for relevant citations. Smaller targeted literature searches were performed to identify pertinent older literature and papers on specific topics and to gather general background information.

CONSIDERATION OF MODE OF ACTION

Much of the committee's task was focused on the mode of action or the toxic and carcinogenic effects of tetrachloroethylene. Because mode of action is considered throughout this report, a brief overview of what it means and of approaches to evaluating it is presented briefly here. The term *mode of action* is defined in the EPA cancer guidelines as a sequence of key events and processes, starting with interaction of an agent with a cell, proceeding through operational and anatomic changes, and resulting in cancer formation. A *key event* is an empirically observable precursor step that is itself a necessary element of the mode of action or is a biologically based marker of such an element. Mode of action is contrasted with *mechanism of action*, which implies a more detailed understanding and description of events, often at the molecular level, than is meant by *mode of action*.

The toxicokinetic processes that lead to formation of the active agent or its distribution to the target tissue, although considered in estimating dose, are not part of the mode of action as the term is used in the guidelines. Examples of possible modes of carcinogenic action are also presented in the guidelines, which state that they include mutagenicity, mitogenesis, inhibition of cell death, cytotoxicity with reparative cell proliferation, and immune suppression.

Understanding of mode of action is crucial for identifying susceptible life stages and determining appropriate approaches to extrapolation beyond the observable dose-response relationships. As a default, dose-response analysis for chemicals whose modes of action are expected to involve mutation involves linear extrapolation. Other modes of action may be modeled with either linear or nonlinear approaches after a rigorous analysis of available data under the guidance provided in the framework for mode-of-action analysis.

In the last decade, a continually evolving framework for considering weight of evidence for hypothesized modes of action and their human relevance has been developed and widely incorporated in guidance and risk assessments for individual chemicals by national and international agencies, including EPA. The framework is relevant to consideration of mechanistic data on both cancer and noncancer effects and sets the stage for informing dose-response relationships through consideration of hypothesized modes of action in the context of key events and their relevance to humans (for example, see Meek 2008). A framework requires delineation of a hypothesis with specified key events and then consideration of the weight of evidence of the hypothesized mode of action in animals in the context of such criteria as consistency, specificity, and biologic plausibility. Human relevance is then taken into account on the basis of consideration of the broader database and such matters as anatomy, physiologic variations, and human disease states.

Recent broad-based acceptance of mode of action and human relevance analyses is a function principally of their value in providing a structured approach to articulation of clear hypotheses, to description of the weight of evidence on which conclusions are based in the context of explicitly stated criteria,

and to delineation of inherent uncertainties. The framework analyses ensure rigor in supporting and communicating the outcome of risk assessment and in facilitating the direction of resources to research to fill critical data gaps. The transparency promoted by framework analyses is expected to contribute to increased consistency in decision-making regarding modes of induction of cancer and later implications for dose-response analysis.

Mode-of-action analyses are based on the assumption that tumors in a single tissue are induced by a single mode of action, although in early stages several (seemingly competing) pathways may contribute. Mode of action is increasingly considered to incorporate toxicokinetics because often the critical first key event (which can be rate-limiting in the context of dose-response relationships) is activation to a toxic metabolite.

ORGANIZATION OF COMMITTEE'S REPORT

In the following chapters, the committee evaluates EPA's presentation and evaluation of the potential adverse health effects of exposure to tetrachloroethylene. Chapter 2 provides a brief overview of the toxicokinetics of tetrachloroethylene because understanding how the body handles tetrachloroethylene is critical for understanding its effects in the later chapters focused on specific organ systems. Chapter 3 presents an evaluation of the neurotoxic effects of tetrachloroethyelene; such effects were the basis of EPA's derivation of the RfC and RfD for tetrachloroetheylene, so the review focuses on evaluating the strengths and weaknesses of available studies and their utility in deriving reference values. Chapter 4 reviews EPA's presentation of the reproductive and developmental toxicity of tetrachloroethylene. That is followed by a chapter on the genotoxicity of tetrachloroethylene, which factors into the consideration of cancers of the liver (Chapter 6), kidney (Chapter 7), hematopoietic system (Chapter 8), and other organs (Chapter 9). Those toxicology reviews are followed by an assessment of EPA's derivation of the noncancer reference values (Chapter 10) and cancer-risk values (Chapter 11). Chapter 12 provides the committee's recommendations for future reassessments of tetrachloroethylene.

2

Overview of the Toxicokinetics of Tetrachloroethylene

It is important to be familiar with the toxicokinetics of tetrachloroethylene when evaluating the Environmental Protection Agency's draft Integrated Risk Information System (IRIS) assessment because many of the chemical's effects are thought to be associated with metabolites rather than with tetrachloroethylene itself. The draft IRIS assessment includes a thorough cataloging of the published literature on tetrachloroethylene metabolism, including consideration of the specific metabolite isoforms that may be involved and polymorphic variants. This chapter presents a brief overview of the absorption, distribution, metabolism, and excretion of tetrachloroethylene to provide context for discussions in this report. More specific toxicokinetic issues associated with specific outcomes and the committee's review of how they are handled in the draft IRIS assessment are discussed in later chapters.

Tetrachloroethylene is a volatile, lipophilic small molecule that is rapidly and extensively absorbed after inhalation and oral exposure. It can also be rapidly absorbed through the skin (Stewart and Dodd 1964), but dermal absorption appears to be a less important route of exposure. In humans, inhalation exposure to tetrachloroethylene typically results, within a few hours of exposure, in a pseudoequilibrium between inspired air and blood although there can be substantial interindividual differences in absorption behavior (Chiu et al. 2007). After oral dosing in animals, peak blood tetrachloroethylene concentrations are typically reached within 15-30 min, and systemic bioavailability is typically greater than 80% (Dallas et al. 1995); once absorbed, tetrachloroethylene is rapidly distributed throughout the body, and well-perfused tissues reach a pseudoequilibrium with blood within a few minutes. For example, after oral administration of a 10-mg/kg dose of tetrachloroethylene in rats, peak tissue concentrations occurred within 10-15 min in blood, brain, heart, lungs, kidneys, and liver (Dallas et al 1994). The elimination half-life of tetrachloroethylene was comparable

among those tissues, between 6 and 7 hours (Dallas et al 1994). In poorly perfused tissues, such as fat and muscle, peak tetrachloroethylene concentrations are reached after a longer delay, which may be an hour or more than a day for adipose tissue. The elimination of tetrachloroethylene from fat is also much slower than that from other tissues and can take twice as long (Dallas et al. 1994). Because of its lipophilicity, the highest concentrations of tetrachloroethylene are found in adipose tissue (Savolainen et al. 1977; Dallas et al. 1994). In humans, the fat-to-blood concentration ratio has been estimated to be as high as 90:1 (Monster et al. 1979). Relatively high concentrations are also observed in the liver and brain (Savolainen et al. 1977). On the basis of animal studies and sparse human data, the brain concentration of tetrachloroethylene is 4-8 times the blood concentration (Dallas et al. 1994; Lukaszewski 1979).

The disposition of an absorbed dose of tetrachloroethylene occurs primarily through pulmonary excretion; metabolism is less important than for other chlorinated solvents, such as trichloroethylene. Mass-balance studies in rats with ^{14}C-labeled tetrachloroethylene indicated that 70% or more of an oral or inhaled dose can be recovered in expired air as the parent compound (Pegg et al. 1979; Frantz and Watanabe 1983). The next most important excreted fraction occurs in urine and feces, which may collectively account for up to 23% of an administered dose. A small portion of the dose (less than 3%) may be converted to CO_2 and exhaled. Most of the radioactivity recovered in urine can be attributed to formation of trichloroacetic acid, a nonvolatile metabolite of tetrachloroethylene that is excreted primarily in urine. That general pattern of disposition of tetrachloroethylene appears to be consistent after both oral and inhalation dosing (Pegg et al. 1979). However, it is important to note that the highest urinary and fecal elimination coincide with lower administered doses of tetrachloroethylene.

Despite the low overall metabolism of tetrachloroethylene compared with other chlorinated solvents, its metabolism has been studied extensively in both human volunteers and laboratory animals, using both in vivo and in vitro techniques. The studies showed that many metabolites are produced, including some known to be cytotoxic, mutagenic or both. Tetrachloroethylene metabolism can be viewed as having three pathways. The first is cytochrome P-450-mediated (CYP-mediated) oxidation. The second and third share a starting point: direct conjugation with glutathione to *S*-(1,2,2-trichlorovinyl)glutathione (TCVG) and then further processing to *S*-(1,2,2-trichlorovinyl)-L-cysteine (TCVC). For the second pathway, β-lyase catalyzes the formation of reactive products from TCVC. The third pathway is independent of β-lyase: TCVC is processed further by acetylation and sulfoxidation reactions. Genotoxic and cytotoxic metabolites are formed by each of these pathways. The predominant metabolic pathway is the CYP path, followed by the β-lyase pathway and then the β-lyase independent pathway. The TCVC derivatives are toxicologically important but quantitatively minor metabolites. A simplified scheme is shown in Figure 2-1.

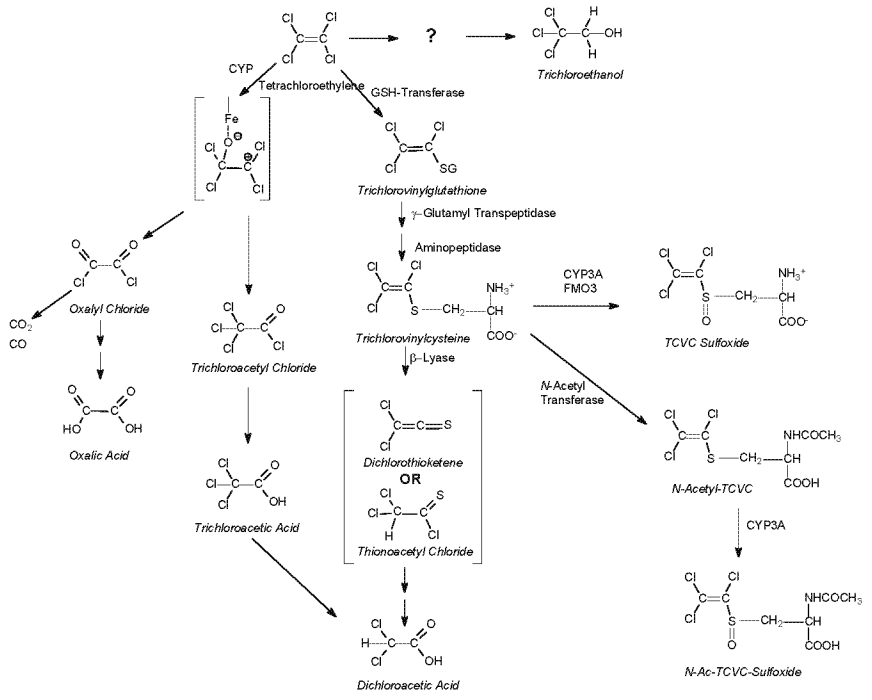

FIGURE 2-1 Simplified illustration of the metabolic pathways of tetrachloroethylene.

THE CYTOCHROME P-450 PATHWAY

The two major products of tetrachloroethylene metabolism by the CYP pathway are trichloroacetyl chloride and oxalyl chloride (Yoshioka et al. 2002). Trichloroacetyl chloride is mutagenic in the Ames test (DeMarini et al. 1994). Trichloroacetyl chloride reacts with lysine on protein to form stable trichloro adducts that can be detected with a specific antibody (Pahler et al. 1998). Trichloroacetyl chloride hydrolyzes to trichloroacetic acid (TCA), which produces liver cancer in mice (Nagano et al. 1998). Oxalyl chloride forms oxalic acid (possibly via oxalyl phosphate) or decomposes to CO_2 and CO. Oxalic acid has long been known to be nephrotoxic; calcium oxalate complexes result in tubular toxicity (Guo and McMartin 2005) and nephrolithiasis (Bushinsky et al. 2008).

Mechanistic studies on the products of CYP oxidation of tetrachloroethylene indicate that trichloroacetyl chloride is the predominant product of the CYP-tetrachloroethylene complex; formation of tetrachloroethylene epoxide is much less favored (Yoshioka et al. 2002). Formation of chloral by rearrangement of tetrachloroethylene epoxide has been postulated, as a pathway to trichloroetha-

nol in analogy with trichloroethylene. Neither chloral nor chloral hydrate has been identified after tetrachloroethylene exposure. Chloral is a product of trichloroethylene oxidation by CYP although not through an epoxide intermediate (Miller and Guengerich 1982). Chlorine migration of the CYP-oxygenated trichloroethylene results in formation of chloral, whereas the product of tetrachloroethylene is trichloroacetyl chloride.

Rats and mice given tetrachloroethylene by gavage were reported to excrete trichloroethanol in urine (Dekant et al. 1986a). The formation of trichloroethanol from tetrachloroethylene has been reported after occupational exposure (Birner et al. 1996), but it was not confirmed in human volunteers exposed to tetrachloroethylene (Volkel et al. 1998; Chiu et al. 2007). Birner et al. (1996) noted that—on the basis of studies by Larson and Bull (1992)—TCA does not undergo reduction to trichloroethanol and could not explain trichloroethanol formation; a later publication from the same group concluded that trichloroethanol was an artifact of trichloroethylene exposure (Volkel et al. 1998).

Small amounts of dichloroacetic acid (DCA) may be produced by dechlorination of TCA (Larson and Bull 1992), but most DCA arises from the β-lyase pathway (Volkel et al. 1998; Dekant et al. 1988).

THE β-LYASE PATHWAY

Tetrachloroethylene is conjugated with glutathione to *S*-(1,2,2-trichlorovinyl) glutathione and is later processed by γ-glutamyl transpeptidase and aminopeptidase to TCVC (see Anders et al. 1988; Lash and Parker 2001). γ-Glutamyl transpeptidase is a brush-border enzyme that is found primarily in the renal proximal tubule and to a lesser extent in the bile canalicular membrane. B-Lyase forms 1-mercapto-1,2,2-trichloroethene, which can tautomerize to dichlorothionacetyl chloride or lose HCl to form dichlorothioketene. Dichloro-thionacetyl chloride and dichlorothioketene both yield dichloroacetic acid (Dekant et al. 1988). Dichlorothioketene reacts with lysine on protein to form stable dichloro adducts that can be detected with a specific antibody (Pahler et al. 1998).

Genotoxicity by the β-lyase pathway is supported by several studies. TCVG induces unscheduled DNA synthesis in mammalian kidney cells, and this response is blocked by inhibiting γ-glutamyltranspeptidase or β-lyase; such inhibition indicates that the genotoxic metabolite arises by the β-lyase pathway (Vamvakas et al. 1989a). The dichlorothioketene adenine and cytosine adducts, formed *in vitro* in organic solvents, do have stability under physiologic conditions and are potential mutagens (Muller et al. 1998a). The chlorofluoro analogue forms adducts with calf-thymus DNA and produces strand breaks. That analogue has chemical properties similar to those of dichlorothioketene; ^{19}Fl was substituted for a Cl to increase the sensitivity of detection (Muller et al. 1998b).

TCVC is cytotoxic to proximal tubule cells (Vamvakas et al. 1989b; McGoldrick et al. 2003). The toxicity is decreased by inhibition of β-lyase with aminooxyacetic acid. Elfarra and Krause (2007) reported potentiation of TCVC

toxicity in rats by aminooxyacetic acid, which provides evidence for a β-lyase-independent mechanism in TCVC toxicity in rats in vivo.

Dichloroacetate is produced primarily through the β-lyase pathway and produces liver cancer in rats.

THE β-LYASE-INDEPENDENT PATHWAY

TCVC undergoes acetylation to its mercapturate *N*-acetyl-TCVC and then sulfoxidation to *N*-acetyl-*S*-(1,2,2-trichlorovinyl)-L-cysteine (*N*-Ac-TCVCS), which is mediated by CYP3A or flavin-containing monooxygenase (FMO). In addition, TCVC undergoes sulfoxidation to TCVC-sulfoxide (TCVCS); this is also mediated by CYP3A or FMO (Ripp et al. 1997).

TCVCS is a more potent nephrotoxicant than TCVC *in* vivo (Elfarra and Krause 2007). TCVC toxicity is increased by inhibition of β-lyase with aminooxyacetic acid (Elfarra and Krause 2007), underscoring the importance of the β-lyase-independent pathway for kidney toxicity. TCVCS mutagenicity appears to be untested. *N*-Acetyl-TCVC is not mutagenic in the Ames test but is more cytotoxic than *N*-acetyl-TCVC, which is mutagenic in the Ames test (Werner et al. 1996).

SPECIES DIFFERENCES

There are important differences between species in the metabolism and toxicity of tetrachloroethylene. Much work has focused on differences between humans and rats, particularly on differences that would influence the human risk of renal cancer that has been observed in rat bioassays. Comparison studies between rats and humans indicate that humans metabolize tetrachloroethylene less than rats; this is based on measurement of metabolites (Birner et al. 1996; Volkel et al. 1998) and on the formation of adducts that are detected by antibodies that are specific for either the CYP-derived trichloro adduct or the dichlorothioketene-derived dichloro adduct (Pahler et al. 1998).

The CYP Pathway

The CYP pathway is the predominant route of tetrachloroethylene metabolism in rats and humans. Plasma albumin adducted with the trichloro derivative, indicating metabolism by the CYP pathway, was found in rats and humans exposed to tetrachloroethylene at 40 ppm for 6 hours. Immunochemical staining was used; the staining of protein from rats was 15-20 times more intense than that of protein from humans (Pahler et al. 1999). Cumulative excretion of TCA in urine was measured in rats and humans after similar controlled exposure to tetrachloroethylene at occupationally relevant concentrations (Volkel et al. 1998). The committee used that data to calculate the ratio of urinary TCA excre-

tion corrected for body mass in rats and humans. TCA excretion by rats was about 23 fold that of humans; or humans excreted about 4.4% of the amount excreted by rats.

The β-Lyase Pathway

Metabolism by the β-lyase pathway results in formation of dichloro protein adducts and DCA. Dichloro albumin adducts were detected in rat, but not human, blood samples after tetrachloroethylene exposure (Pahler et al. 1999). Even after immunoaffinity-column enrichment, the dichloro adduct was not detected in human samples. DCA is a stable product of the β-lyase pathway and is excreted in urine. Rats excreted DCA in urine at about one-tenth the amount of TCA, but DCA was not detected in urine collected from human volunteers after exposure to tetrachloroethylene (Volkel et al. 1998). That outcome is consistent with the lower activity of β-lyase in humans (McGoldrick et al. 2003).

The β-Lyase-Independent Pathway

Protein adducts resulting from the β-lyase-independent pathway have not been reported. *N*-Acetyl-TCVC, the mercapturate, is excreted in urine. Volkel et al. (1998) also measured urinary excretion of *N*-acetyl-TCVC after similar exposure to occupationally relevant concentrations of tetrachloroethylene. The Committee calculated the ratio of cumulative urinary excretion of *N*-acetyl-TCVC by rats to be about 5.5 fold that of humans; or humans excreted about 20% of the amount of *N*-acetyl-TCVC excreted by rats. Both rats and humans excrete much more TCA, the CYP-pathway product, than *N*-Ac-TCVC, but the ratio of *N*-acetyl-TCVC to TCA in humans is about 5 fold that of rats. That is, humans excrete relatively more tetrachloroethylene metabolites as *N*-Ac-TCVC than rats. That, too, is consistent with the lower activity of β-lyase in humans (McGoldrick et al. 2003); relatively more TCVC is metablized by the β-lyase-independent pathway in humans.

3

Neurotoxicity

This chapter reviews information presented in the Environmental Protection Agency (EPA) draft Integrated Risk Information System (IRIS) assessment of the effects of tetrachloroethylene on the nervous system. It considers first the human evidence, including an evaluation of EPA's selection of the most critical study on which to base its reference values, and then the evidence from experimental animal studies. The implications of the committee's evaluation on the derivation of EPA's reference values for tetrachloroethylene are discussed in Chapter 10.

HUMAN STUDIES

The epidemiologic studies available for evaluating the neurotoxic effects of tetrachloroethylene were generally cross-sectional. Only one study (Gobba et al. 1998) had outcome measures at two times. Although the cross-sectional study design is limited in establishing temporality in a causal association, the combination of the results of such studies with other information can help to establish an exposure-effect relationship.

In evaluating the human evidence, the committee applied several criteria for determining which studies were the most useful in establishing a reference concentration (RfC) for tetrachloroethylene. The criteria included three general characteristics: the validity of individual studies, the internal consistency of results (for example, Is there an association in the low-exposure group but not in the high-exposure group?), and the consistency of the findings with what is known from other sources (how the study fits into the overall picture of what is known). In selecting studies, the committee considered the target population, the study population, potential confounders, and possible selection or information biases. Statistical issues were also considered. Each study was looked at in the light of those factors, and studies were neither chosen nor rejected on the basis of their results. The selection criteria included consideration of the following factors and questions:

- **Populations:** Are the target and study populations well defined and described? Is the referent group representative of either the unexposed population (in a cross-sectional or cohort design) or of the source population (in a case-control design)? Studies with an inappropriate referent population were given less weight.
- **Selection of participants:** Are the methods for recruiting and enrolling study participants well described? Is there evidence of selection bias? If so, have the authors provided information on the magnitude of the bias? Whether an "effect" is observed in the exposed group is strongly influenced by the choice of the comparison or control group. Thus, the selection and composition of the comparison group is extremely important and in part determines the internal validity of the study. In some cases, there were clear selection biases (for example, selecting comparison groups for the exposed group that did not represent the counterfactual example). That introduces the possibility of selection biases that could easily create the appearance of differences, especially subtle ones, when differences do not exist.
- **Exposure assessment:** How well do the measurements used characterize tetrachloroethylene exposure? How are exposure groups defined? If individual exposure data were available, were they used, or was assignment to exposure groups based on ecologic criteria? In most cases, exposure was estimated at the time of a study. If it is assumed that exposure has only acute, reversible effects, cross-sectional studies are more appropriate. However, if occurrence of an effect when exposure concentrations are low requires long-term exposure, it is important to consider past exposure as well. Exposure assessment ranged from biologic measurements of tetrachloroethylene exposure to environmental exposure assessments. Studies that included measurements and analyses of exposure at the individual level were given greater weight.
- **Assessment of neurologic outcomes:** The end points that were measured in terms of relevance to the visual system and the degree to which the measures are influenced by cognitive function were considered. Studies that used less sensitive measures were given less weight, as were studies that used outcome measures that were more susceptible to observer bias or potential individual confounders (such as ability to follow instructions).
- **Confounding:** Observational studies are always subject to confounding when the exposed and referent groups are imbalanced with respect to factors that are not a result of the exposure but that are also related to the outcome. The committee considered the potential for differences in age, education, learning disabilities, and other variables to confound associations. If the potential for confounding was present and the effects of the confounding were not addressed by the study design or analytic methods, the results of the study were considered to be less credible.
- **Statistical analysis:** Statistical issues were considered, particularly whether the sample size was adequate and whether the approach to analysis was appropriate. Did the studies provide adequate information about the distribution

of exposure levels or results of outcome testing? Were the results influenced by only a few extreme values? If so, was that considered? If continuous data were available, were they used or collapsed as a binary variable, making dose-response analysis or assessment of thresholds impossible? Were tests for interaction of tetrachloroethylene exposure with other variables done? If so, were they properly interpreted?

Having applied those criteria, the committee disagreed with EPA's selection of the study by Altmann et al. (1995) as the critical study on the basis of which exposure limits should be estimated. EPA selected Altmann et al. (1995) because the data in it represent an environmental rather than an occupational exposure and because a standardized computer-assisted testing battery was used. Although those are reasonable considerations, they are not the most relevant for selecting a critical study. The committee concluded that the validity of the results of Altmann et al. (1995) was seriously compromised by the following methodologic deficiencies.

1. The reference group was inappropriate, because it did not represent the counterfactual example. The reference group included employees of the Public Health Office or the Medical Institution of Environmental Hygiene, none of whom resided at their place of employment and who may have lived outside the commercial city center. Personal characteristics as well as differences in exposures in the ambient environment may have confounded the analyses of exposure and neurobehavioral outcomes. Evidence of this selection bias is that although matched by age and sex, the referent group was clearly more educated than the exposed group. The distribution of the 14 exposed participants in the low, medium, and high education categories was four, eight, and two, respectively, and that of the 23 controls, one, 12, and 10. The effect of these differences on the study results could not be evaluated, however, because the numbers of years of education represented in the categories were not provided. Adjusting for education with broad categories rather than years of education is not adequate and can easily result in residual confounding by education. Evidence for residual confounding by education can be seen in the variability of results reported by Altmann et al. (1995) depending on the outcome measure. For example, no association between tetrachloroethylene and visual evoked potentials (VEPs) was found. That is important because changes in the visual system and abnormalities in VEPs have been associated with exposure to tetrachloroethylene and chemically related solvents (Bushnell and Crofton 1999; Gobba 2003; Bushnell et al. 2007; Benignus et al. 2009) and selected organic solvents (Benignus et al. 2009) and are unrelated to education. Measures of vigilance, attention, and visual memory are strongly associated with education and premorbid intelligence (Lezak et al. 2004). Those measures showed poorer performance in the exposed group, whereas measures of eye-hand coordination and finger tapping, which are weakly related to education and premorbid intelligence, were similar in the two groups.

2. The Neurobehavioral Evaluation System (NES) battery used to assess brain dysfunction related to exposure appropriately included four subtests that have been shown in other research to be associated with solvent exposure. However, the battery has no norms for this population, and some of the tests have not been well validated with regard to what they reveal about brain damage from any cause. The absence of norms makes it especially important to have standardized measures of intellectual function that can be used to characterize the native intellectual capacity of the two groups. Examples of such tests are the NES Vocabulary subtest, the Wide Range Achievement Test Reading subtest, and the Wechsler Adult Intelligence Scale Information subtest. Tests of native intellectual function like those are important to include in a battery used to assess neurocognitive outcomes because they are resistant to the effects of central nervous system insults from neurotoxic exposure. They can be used to control statistically for differences in premorbid function between exposed and control groups. Failure to use such measures can cause investigators to conclude that measured group differences in cognitive function are due to exposure when in reality they might exist without any exposure.

3. The authors indicated that there were 92 potentially eligible subjects, of whom 19 were selected as participants. It was unclear whether the 19 were selected because they were the only ones who had blood tetrachloroethylene over 2 μg/L, lived next to a dry-cleaning facility for at least 1 year, and had no occupational exposure to organic solvents. Even though a blood tetrachloroethylene concentration of over 2 μg/L was required for entry into the study, no concentrations were reported for five subjects (subjects 10-14) taken in their apartments (Figure 1A of Altmann et al. [1995]). Without those specifications, it is impossible to determine whether the sample was biased (that is, whether others were excluded for reasons other than study design).

4. Tetrachloroethylene was measured in air samples from homes for 7 days. Figure 1B of the paper purports to show indoor air concentrations for exposed participants and controls, but no concentrations are shown for the referent group. For subject 13 of the exposed group, there was no indoor air measurement, there was no tetrachloroethylene concentration in blood drawn in the apartment, and the blood concentration obtained at the time of testing was at the limit of detection (0.5 μg/L). Duration of residence of the 14 exposed ranged from 1 to 30 years; only mean duration was reported, not median. Given only a mean value, there is no way to know whether most of the exposed subjects had relatively short exposures and just a few had long exposures. The amount of time that residents spent in their apartments is unknown. Time out of the apartments before neurobehavioral testing was unknown but was believed to account for the lower blood tetrachloroethylene concentrations before testing. Two exposed subjects had blood tetrachloroethylene concentrations at the limit of detection when tested, whereas the blood concentrations of subject 4 were 30 μg/L in the apartment and 200 μg/L at the time of testing.

5. In the analyses, exposure is defined by group membership (yes or no) rather than by individual markers of exposure, so a dose-effect relationship could not be assessed. As stated above, group differences in neurobehavioral performance were more likely to be related to residual confounding by education or pre-exposure intellectual capacity than to exposure.

Another paper cited in the draft IRIS assessment that associated environmental tetrachloroethylene exposure with visual-contrast sensitivity (VCS) dysfunction reported on a pilot study by Schreiber et al. (2002). The study also suffered from important methodologic problems that limit its usefulness, including the criteria used to select the exposed group, selection of a noncomparable referent group, and errors in analysis and interpretation. It has been suggested that the significant results reported by Schreiber et al. were influenced largely by two exposed children who had diagnoses of developmental disorders (Storm and Mazor 2004). The total sample in the study was 17, of whom four were children; when the children were excluded from analyses, no significant associations were observed. Given the cross-sectional design of the Schreiber et al. study, it cannot be determined whether exposure preceded the developmental disorders. The small sample makes results highly sensitive to a few observations.

The published papers that the committee judged to be more appropriate to use as a point of departure for derivation of the RfC and reference dose (RfD) were Echeverria et al. (1995), Cavalleri et al. (1994) in combination with Gobba et al. (1998) and Altmann et al. (1990). The reasons for the selections are given below.

Echeverria et al. (1995) conducted a well-designed study of the relationship between acute and cumulative tetrachloroethylene exposure in dry-cleaning shops in Detroit, Michigan, and performance on a neuropsychologic battery. There was no "unexposed" group, but the referent group (lowest exposed; mean air tetrachloroethylene concentrations, not greater than 11.4 ppm) was in the same cohort of dry-cleaning shops as the "exposed" group (mean air tetrachloroethylene concentrations, not greater than 40.8 ppm). Using an internal referent group reduced the potential for the types of selection bias present in many other studies. In the analyses, several potential confounders were considered, including, age, education, verbal skill, alcohol consumption, and prior intoxicant exposure. The authors used a stepwise selection procedure for adjustment, but it is not clear which variables were ultimately used. After adjustment for the covariates, performance on tests for Wechsler Memory Scale Visual Reproduction, NES Pattern Memory, and NES Pattern Recognition was significantly poorer in workers who had a high index of lifetime tetrachloroethylene exposure than in workers who had a low index of lifetime tetrachloroethylene exposure (Table 3-1). Estimated lifetime tetrachloroethylene exposure was positively associated with self-reported "tension" (on the Profile of Mood States) and inversely associated with NES Pattern Recognition scores. Subanalysis

demonstrated some similarity in the test results affected by tetrachloroethylene and alcohol consumption: Visual Reproduction, Pattern Memory, and Pattern Recognition. This similarity underscores the importance of adjusting for alcohol use in analyses of effects of tetrachloroethylene. The study is not without limitations in that recruitment was influenced by the lowering of the permissible exposure limit from 50 ppm to 25 ppm and by owners' emphasizing the cost of such a change for relatively little effect on health status; therefore, only 23 of a potentially eligible 125 shops participated, for a total of 65 exposed workers.

Cavalleri et al. (1994) examined color-vision loss in 35 dry-cleaning workers in 12 small dry-cleaning shops in Modena, Italy, and in controls who had no solvent exposure and were matched by age, sex, alcohol use, and cigarette-smoking. Inclusion criteria were "apparently healthy," average daily alcohol intake under 50 g/day, smoking fewer than 30 cigarettes/day, and corrected visual acuity of at least 6/10. Color vision was evaluated with the Lanthony 15 Hue desaturated panel, which was repeated 10 times. Few exposed or control workers were able to perform the test without error. Results wereexpressed as a color-confusion index (CCI) with errors in blue-yellow color vision. Tests were performed monocularly, and the mean CCI for both eyes was used in the analyses, although CCI may be affected in only one eye after tetrachloroethylene exposure. Air tetrachloroethylene concentrations obtained with personal passive sampling for 1 day produced a mean time-weighted average (TWA) for drycleaners of 7.27 ± 8.19 ppm (range, 0.38-31.19 ppm). The mean CCI for the drycleaners was significantly higher (1.192 ± 0.133) than that of controls (1.089 ± 0.117). The statistically significant relationship between TWA of tetrachloroethylene exposure and CCI depended on two extreme values. CCI was not related to duration of exposure or to an integrated index of exposure; only current exposure was known, and there were no data on tetrachloroethylene concentrations in previous years. The study established the protocol and baseline for the Gobba et al. (1998) study 2 years later, which was of greater interest to the committee.

TABLE 3-1 Estimated Mean[a] Neuropsychologic Test Results by Lifetime Exposure to Tetrachloroethylene in Study by Echeverria et al. (1995)

	Exposure Group		
Test	Low (N = 24)	Moderate (N = 18)	High (N = 23)
Visual reproduction	9.4 ± 1.21	8.9 ± 1.24	8.08 ± 1.24
Pattern memory	10.51 ± 0.82	10.36 ± 0.75	9.70 ± 0.72
Pattern recognition	14.39 ± 0.49	13.97 ± 0.49	13.83 ± 0.70
Tetrachloroethylene concentration at testing, ppm	< 0.6	4.3–12.1	11.4–41.8

[a]Means adjusted for covariates ± standard deviation.

Gobba et al. (1998) re-examined 33 of the workers from the Cavalleri et al. study for color-vision loss after an interval of 2 years. This study was unique in that it examined the same workers at two times. Overall, tetrachloroethylene concentrations remained unchanged for the whole group, but 19 workers (group A) had exposure to significantly increased tetrachloroethylene concentrations at the time of the second assessment, and the remainder (group B) had exposure to significantly lower concentrations because of changes in the processes used in their dry-cleaning shops. Demographic information was provided on the group as a whole but not the two subgroups. The mean CCI increased significantly over the 2 years in group A (from 1.16 ± 0.15 to 1.26 ± 0.18) but remained unchanged in group B (1.15 ± 0.14 and 1.15 ± 0.13). In comparison, the control group from the Cavalleri et al. study, which was not re-examined in the Gobba et al. study, had a mean CCI of 1.08 ± 0.10. The clinical significance of these CCI changes is uncertain. The participants in the Gobba et al. study had exposure concentrations closer to those reported in environmental studies. That the CCI did not improve in the group with lower tetrachloroethylene exposure might be because improvement in workplace conditions had been in place for only a short time or because the visual changes are not reversible.

Altmann et al. (1990) randomly allocated 22 healthy young male subjects to exposure to tetrachloroethylene at 10 ppm or 50 ppm in a chamber for 4 hours on 4 consecutive days, and blood samples were taken for tetrachloroethylene testing and visual and neurophysiologic tests were performed. All subjects had normal visual acuity and no previous solvent exposure. Increased latency in VEPs was observed in subjects exposed to tetrachloroethylene at 50 ppm, and decreased latency at 10 ppm; the greatest effect was observed on the last day of exposure. VEPs with the smallest visual angle and on the last day of exposure provided the greatest intergroup differences. VCS tests on five subjects (two at 50 ppm and three at 10 ppm) showed improvement at the low and intermediate spatial frequencies in the 10-ppm group but loss in the 50-ppm group. Brainstem auditory evoked potentials were not associated with tetrachloroethylene exposure. The lowest observed-adverse-effect level (LOAEL) appeared to be 10 ppm for VEP outcomes.

A second paper (Altmann et al. 1992) published on the above study summarized data on neurobehavioral outcomes but is not recommended for use in determining reference values. Performance during 4 days of exposure was compared with performance obtained on day 1 in the chamber, when there was no exposure. The NES subtests measuring mood and "cognitive function" showed no decrement in performance with days of exposure, but the continuous performance test, tracking task (hand-eye coordination subtest), and simple reaction time task showed improvement over time that was more pronounced in the 10-ppm control group than in the 50- ppm exposure group. However, the measure of premorbid function used in the study (a vocabulary test) was not included as a control measure in the data analyses; it might have affected the outcomes on all NES subtests, especially those of learning and memory. Some NES subtests were given only twice and some at every session; it is not clear which were

given when, but it might have influenced which test outcomes had significant results because of differences in practice effects.

ANIMAL STUDIES

This section describes controlled-exposure studies of experimental animals. As noted in the draft IRIS assessment, most animal studies have involved inhalation exposures to tetrachloroethylene at concentrations of about 30 ppm to over 1,000 ppm or administration by noninhalation routes of tetrachloroethylene at 100-to 4,000 mg/kg. Because of the relevance of the exposure regimen, the inhalation studies are emphasized here. However, it should be noted that studies like that of Warren et al. (1996) and Moser et al. (1995) deliver a known amount of tetracholorethylene by other routes (for example, by gavage) and also support tetrachloroethylene's neurotoxicity. Warren et al. reported effects on a refined end point, schedule-controlled behavior, and linked behavioral deficits to blood and brain concentrations. Moser et al. (1995) used a broad range of doses administered acutely or "sub-acutely" (14 days) and reported LOAELs and no-observed-adverse-effect levels (NOAELs) on a well-characterized Functional Observational Battery.

Incorporating the animal literature into an assessment of tetrachloroethylene's neurotoxicity has several advantages. The animal literature can demonstrate the plausibility of claims that neurotoxicity occurs, identify the role of dose and duration of exposure in neurotoxicity, discover neurotoxic effects for further study in humans, confirm with controlled exposures that neurotoxicity occurs in a specific domain, link effects to tissue concentrations, and determine mechanisms of action and similarities and differences between other compounds in the same class. The animal studies entail known histories and living conditions and controlled exposure conditions, usually over a range of doses or concentrations; this allows assessment of dose-effect relationships under conditions that are less influenced by the covariates and biases that hamper the interpretation of human exposures.

The literature describing controlled acute and subchronic inhalation exposures of laboratory animals is summarized in the EPA document. The end points affected include neurotransmitter or neurochemical concentrations (Honma et al. 1980; Nelson et al. 1979; Briving et al. 1986; Karlsson et al. 1987), long-chain fatty acid concentrations (Kyrklund et al. 1984, 1987), RNA expression (Savolainen et al. 1977), DNA expression and brain weight (Rosengren et al. 1986; Wang et al. 1993), electrophysiologic measures and evoked potentials (Mattsson et al. 1998), and locomotor activity (Savolainen et al. 1977; Kjellstrand et al. 1985; Szakmary et al. 1997), all of which indicate tetrachloroethylene's neurotoxcity. Some studies published after the draft IRIS assessment was written have applied physiologically based pharmacokinetic (PBPK) modeling to characterize not only the dose to which an animal is exposed but the concentration at the target tissue for neurotoxicity, the brain (e.g,, Boyes et al. 2009).

The incorporation of PBPK modeling will facilitate generalization among species and among routes of exposure. The process can contribute to the identification of mechanisms and modes of action and can enhance understanding of the comparative toxicity of different solvents.

The animal studies have limitations. Most notably, as in the studies of controlled human exposure, they use concentrations that are much higher and durations that are much shorter than those experienced environmentally or occupationally. Incorporating their results into a risk assessment must entail the application of uncertainty factors to identify hazard at environmentally, or even occupationally, relevant concentrations. In addition, the dependent measures in most studies differed from those identified in the human literature as particularly sensitive to tetrachloroethylene exposure. In contrast, recently published papers, such as those by Oshiro et al. (2008) and Boyes et al. (2009), use end points that are directly relevant to humans.

The draft IRIS assessment reviews two papers by Kjellstrand et al. (1984, 1985 [see Table 4-6, page 4-409 of EPA 2008]) for neurotoxicity. However, the 1984 study is not appropriate for assessing neurotoxicity; its strengths are that it involved doses that ranged from 9 to 3,600 ppm and durations that ranged from 1 to 120 days and continuous exposure or exposure for a different number of hours per day, but no central nervous system end points were examined. EPA reports that brain butyrylcholinesterase activity was affected, but plasma was analyzed, so the relevance to neurotoxicity is unclear. Some mice were examined for locomotor activity, but exposure and effects are poorly described and unusable. Although the exposure was acute, the relationship between locomotor activity and exposure is described better in the 1985 paper.

Overall, the animal studies support the conclusion that tetrachloroethylene is neurotoxic, but, except for the study by Mattsson et al. (1998), the end points used in the animal studies that were reviewed by EPA were nonspecific and not directly related to the visual or cognitive effects reported in the human literature. The studies therefore provide only indirect support for EPA's conclusions. The studies by Mattsson et al. entailed exposure 6 hours/day 5 days/week for 13 weeks and examined VEP and other functional effects, so their results are directly pertinent to human exposures. A NOAEL and a LOAEL were identified. Several related reports have been published since the draft IRIS assessment was written (for example, Boyes et al. 2009; Oshiro et al. 2008); they describe dose-effect relationships, spanning a broad range of doses, between acute exposure and visual and signal-detection end points.

In the Boyes et al. (2009) study, rats were exposed head-only to tetrachloroethylene while VEPs were recorded. Exposures were to concentrations of tetrachloroethylene ranging from 1,000-4,000 ppm for 1-2 hours, using concentration and time combinations derived from kinetic analyses. The most sensitive end point was the F2 (frequency-doubling) component of the evoked potential spectrum, a measure thought to reflect the activity of cortical neurons that respond to both stimulus offset and onset. Boyes et al. also conducted a toxicokinetic analysis relating exposure concentration (250-4,000 ppm) and duration (1

hour followed by a 6-hour washout period) to brain concentration. From this analysis, the investigators were able to link brain concentrations of tetrachloroethylene to visual function and to estimate an ED_{10} of 0.68 mg/L and ED_{50} of 47 mg/L.

In the study by Oshiro et al. (2008), rats were exposed by inhalation to tetrachloroethylene at 500, 1,000, and 1,500 ppm for 1 hour, during which a visual signal detection task was performed. Rats were trained to indicate the occurrence or nonoccurrence of a light flash during a trial period that lasted from 0.3 to 24.39 seconds, and individual trial durations were random. Exposure to tetrachloroethylene did not change the number of "correct" detections, but significantly increased the number of times that the rats incorrectly indicated a signal (false alarm), increased response time, and decreased the number of trials completed. The false-alarm rate was affected at the lowest concentration (500 ppm) and a NOAEL was not identified. The authors concluded that the results suggest attention deficits.

EPA also reviewed animal studies conducted with intraperitoneal or oral exposure. The studies of exposure of adults included functional observational batteries (Moser et al. 1995), locomotor activity (Fredriksson et al. 1993; Motohashi et al. 1993), and schedule-controlled operant behavior (Warren et al. 1996). EPA did not use the studies in establishing an oral RfD for chronic adult exposures, because effects occurred at high doses (150 mg/kg per day or higher) in the well-controlled studies.

The mode of action for tetrachloroethylene's neurotoxicity is discussed in a separate section of the draft IRIS assessment (Section 4.6.4). The assessment notes that while the mechanism by which tetrachlorethylene acts is unknown, the evidence is good that it acts on ligand-gated ion channels like other organic solvents. EPA correctly notes that solvents act similarly to ethanol on $GABA_A$ receptors and that there are orderly structure-activity relationships, but the citation in support of this observation (Mihic 1999) reviews ethanol and not other solvents. As implied in the IRIS assessment, tetrachloroethylene's effects on brain fatty acids are interesting but its functional significance is not clear. A weakness of the IRIS assessment's treatment of the evidence on tetrachloroethylene's mechanism of neuorotoxic action is that it is entirely descriptive and isolated from the rest of the document. Specifically, the implication that it resembles other volatile organic solvents is not used elsewhere in the document in support of tetrachloroethylene's toxicity to the adult or the developing nervous system. In light of the importance of neurotoxicity to the development of the RfC, this is surprising.

DEVELOPMENTAL NEUROTOXICITY

The literature on developmental neurotoxicity is limited. EPA's discussion of this important issue is distributed between the sections on neurotoxicity and reproductive toxicity. In light of the sensitivity of the developing nervous system

to neurotoxicants, including solvents (Costa et al. 2004; Grandjean and Landrigan 2006; Slikker 1994), the topic should have been given separate treatment. The EPA document appropriately raises concerns that the studies of tetrachloroethylene-exposed children are small or sufficiently problematic that firm conclusions cannot be drawn from them. Several effects have been reported, including alterations in sensorimotor function (Nelson et al. 1979; Umezu et al. 1997), brain neurochemistry (Nelson et al. 1979), and locomotor activity (Fredriksson et al. 1993; Motohashi et al. 1993; Nelson et al. 1979; Szakmary et al. 1997). Some of these studies used very high concentrations, but others involved concentrations relevant to potential human exposures.

Nelson et al. (1979) exposed pregnant rats to tetrachloroethylene at 900 ppm on gestational days 7-13 or 14-20 or at 100 ppm on days 14-20. No significant tetrachloroethylene-related effects were reported in the animals exposed at 100 ppm, but effects were noted in those exposed at 900 ppm. The tetrachloroethylene-exposed dams consumed less feed and gained less weight than air-exposed controls. No significant differences in growth were noted in offspring, but the draft IRIS assessment incorrectly states that diminished weight gain in offspring was reported. Offspring showed deficits in neuromuscular and sensorimotor functions and increases in locomotor activity.

Fredriksson et al. (1993) also reported changes in locomotor activity in 60-day-old rats after oral exposure to tetrachloroethylene administered (at 5 and 320 mg/kg) on postnatal days 16-20; the effects were not dose-related. The draft IRIS assessment appropriately raised a concern about adequate control for litter effects in the study. It is widely accepted that litter effects must be controlled for in analyses of developmental exposure. Usually litter effects are handled by including only one pup, or one pup per sex, from each litter in studies of prenatal or perinatal exposures. That is, to avoid "litter effects," the litter should be the statistical unit. A failure to follow that convention inflates the type I error rate. Fredriksson et al. (1993) did not follow it but instead assigned pups to treatment groups randomly, so some treatment groups contained siblings. Some of the authors of the paper have argued that their approach is appropriate and does not inflate the type I error rate (Ericksson et al. 2005); their discussion is also cited in the draft IRIS assessment. Because exposures took place on postnatal days 16-20, the extent to which litter effects confounded the results in the 1993 Fredriksson et al. study is unclear. Nonetheless, the absence of a dose-effect relationship is of concern.

In a short communication, Kyrklund and Hagid (1991) described changes in brain fatty acids of neonatal guinea pigs exposed to tetrachloroethylene at 160 ppm during gestation, but the samples were very small, and many important details were lacking. As noted in the draft IRIS assessment, there was evidence of litter effects in this study, and EPA correctly notes that there are concerns about the absence of a dose-effect relationship and of important methodologic considerations, such as use of non-blinded observers on end points that involved subjective observations and difficulty in relating intraperitoneal routes of administration to oral or inhalation routes.

As noted in the draft IRIS assessment (section on "Mode of Action for Neurotoxic Effects" [4.6.4]), tetrachloroethylene has much in common with other volatile organic solvents, anesthetics, and alcohols. These shared mechanisms, coupled with similarities in the kinetics of these compounds and the high vulnerability of the developing brain to organic solvents and alcohols, raise concerns about the vulnerability of the developing organisms to tetrachloroethylene. The material on developmental neurotoxicity, while identifying the studies directly pertinent to tetrachloroethylene, omits mention of evidence that might be derived from similarly acting compounds. A separate section might have addressed these issues more thoroughly.

FINDINGS AND RECOMMENDATIONS

EPA's selection of neurotoxicity with emphasis on the outcomes of cognitive and visual dysfunction in adults is appropriate as an end point for deriving a point of departure for development of its reference values. However, the committee disagrees with EPA that the study by Altmann et al. (1995) should be the basis for the noncancer risk values. The committee recommends the use of studies by Altmann et al. (1990), Cavalleri et al. (1994) as a baseline for Gobba et al. (1998), and Echeverria et al. (1995). A new animal study by Boyes et al. (2009) also provides a strong basis for a point of departure. Those five studies provide a stronger scientific basis for deriving the RfC and RfD. Despite the importance of the developing nervous system, the literature on potential neurodevelopmental effects is not sufficient to support the derivation of an RfC. This does not mean that developmental neurotoxicity is unlikely. The broader solvent literature raises significant concern about potential developmental neurotoxicity. While the draft IRIS assessment notes that tetrachloroethylene enters the developing brain, it appears to dismiss the potential for developmental neurotoxicity independent of reproductive or maternal toxicity.

Additional research may help to fill gaps in the evidence. For example, studies of developmental neurotoxicity are needed to fill an important gap in the database on tetrachloroethylene. Well-designed epidemiology studies of tetrachloroethylene and neurological end points that characterize both past and current exposure would be helpful. These studies should be done in populations with a range of exposures (such as occupational studies with a wide distribution of exposure and environmental exposures via both air and water).

4

Reproductive and Developmental Effects

The Environmental Protection Agency (EPA) draft Integrated Risk Information System (IRIS) assessment describes the key animal developmental-toxicity and reproductive-toxicity studies of tetrachloroethylene in Section 4.7.2 and provides useful summaries of the study results in its Tables 4-8 and 4-10. In evaluating the studies described by EPA, the committee applied several criteria to determine whether there is sufficient evidence to identify tetrachloroethylene as a reproductive or developmental toxicant in animals and to identify a reference concentration based on reproductive or developmental end points. The criteria included consideration of identification of adverse effects that were not confounded by excessive maternal toxicity, use of multiple experimental exposures, identification of a no-observed-adverse-effect level (NOAEL), and conformity with current regulatory testing guidelines.

The committee agrees with the NOAEL of 100 ppm based on the study by Tinston (1994). EPA'a derivation of a comparative reference value (RfV) based on reproductive or developmental toxicity is an important addition to the toxicologic information on tetrachloroethylene and will be helpful in assessing potential health risks related to these end points. However, EPA's rationale for selecting the Tinston (1994) study instead of the Carney et al. (2006) study for the benchmark dose analysis and derivation of the RfV is not presented in the document and therefore is unclear. A major criticism of Section 4.7.2 has to do with the general lack of transparency regarding the critical analysis that EPA conducted of the studies described. The strengths and limitations of individual studies are not adequately discussed, and evaluations of reported maternal toxicity and comparisons of studies that yielded supporting or conflicting evidence of developmental or reproductive toxicity are not adequate. As a result, the reader cannot readily conclude that EPA had sufficient data for a risk assessment. Furthermore, the scientific basis for considering some studies and not others for derivation of a comparative RfV based on reproductive or developmental toxicity is not apparent. EPA does not state whether the experimental animal evidence of tetrachloroethylene-induced developmental toxicity and reproductive

toxicity is sufficient or insufficient on the basis of criteria in its risk-assessment guidelines. Some of the specific deficiencies in Section 4.7.2 are described below.

LIMITATIONS OF THE DATABASE

Information analogous to that on page 4-124 of the draft IRIS assessment, which discusses general limitations of the human reproductive-toxicity and developmental-toxicity studies, would be useful. It would provide a context for the descriptions of individual studies and would be helpful in characterizing the animal developmental-toxicity and reproductive-toxicity data available for hazard identification and dose-response evaluation. For example, only two studies of the reproductive toxicity of tetrachloroethylene are described, and many of the developmental-toxicity studies described have limitations. The limitations include use of a single exposure level, insufficient study details, excessive maternal toxicity, and lack of conformity with current EPA and Organisation for Economic Co-operation and Development (OECD) regulatory testing guidelines because of when the studies were conducted.

COMBINED DISCUSSION OF REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

EPA discusses the evidence on reproductive toxicity and developmental toxicity together. Without a separate discussion of each, it is difficult to identify conflicting data and data gaps and to assess whether there is sufficient evidence of toxicity for each end point according to the criteria in the EPA (1991, 1996) guidelines. The sequence or order in which the studies are described in Section 4.7.2 complicates the issue. The two studies that provide specific information on the reproductive toxicity of tetrachloroethylene, Tinston (1994) and Beliles et al. (1980), are not discussed sequentially. The end-point-specific evidence from the well-conducted Tinston (1994) reproduction study and the Carney et al. (2006) developmental-toxicity study is either not stated or not emphasized by EPA. For example, EPA does not conclude from the Tinston (1994) two-generation reproduction study that tetrachloroethylene had no significant effect on reproductive performance or fertility in rats at up to 1,000 ppm. The results of the Beliles et al. (1980) study, which showed that tetrachloroethylene at 500 ppm had no significant effect on the sperm of rats, are consistent with the adverse effect on fertility in the Tinston study, but the relationship of this finding to the Tinston (1994) study is not discussed. The Summary on page 4-134 does not mention the results of the Carney et al. (2006) developmental study, which showed that tetrachloroethylene at 249 ppm, in the absence of maternal toxicity, can produce developmental toxicity in rats (reduced fetal and placental weights and incomplete ossification of thoracic vertebral centra).

EVALUATION OF THE RELATIONSHIP OF MATERNAL AND DEVELOPMENTAL TOXICITY

The EPA risk-assessment guidelines (EPA 1991, p. 18) state: "Since the final risk assessment not only takes into account the potential hazard of an agent, but also the nature of the dose-response relationship, it is important that the relationship of maternal and developmental toxicity be evaluated and described." It is not clear whether EPA evaluated the range of maternal-toxicity data (mild to severe effects) that are reported in the studies described, inasmuch as interpretation of the data with regard to the developmental toxicity of tetrachloroethylene is not presented. For example, in the Schwetz et al. (1975) study, tetrachloroethylene produced a statistically significant increase in resorptions and mild, statistically significant maternal toxicity (4-5% reductions in mean maternal body weight compared with controls) in rats. Food consumption and liver weights were not affected by tetrachloroethylene exposure. Maternal toxicity is listed in the EPA draft's Table 4-8 as an "Effect," but there is no discussion of its relationship to the increased resorptions. According to the EPA risk-assessment guidelines, the increased resorptions in the Schwetz et al. (1975) study represent tetrachloroethylene-induced developmental toxicity in that they were produced at doses that caused minimal maternal toxicity. Maternal toxicity (decreased body weight gain and increased liver weight and serum enzyme activities) at tetrachloroethylene concentrations of 221, 664, and 1,254 ppm is also listed as an "Effect" in Table 4-8 for the Szakmary et al. (1997) study. EPA does not point out that the excessive maternal toxicity at 664 and 1,254 ppm (decreases of 37% and 40% in maternal body-weight gain, respectively, compared with 13% at 221 ppm) makes the developmental effects (such as skeletal retardation and decreased fetal weight) difficult to interpret and of limited value on the basis of its risk-assessment guidelines.

STUDY STRENGTHS AND LIMITATIONS AND CONSISTENCY OF RESULTS

Section 4.7.2 of the EPA draft does not identify the studies that are scientifically strong and the studies that are weak. Supportive and conflicting studies in the database also are not adequately identified. For example, EPA does not explain why confidence in the Tinston (1994) and Carney et al. (2006) studies should be higher than in the other studies described. In addition to being well conducted, both Tinston and Carney et al. have multiple experimental exposures, report effects associated with lower exposures that are not confounded by excessive maternal toxicity, and identify NOAELs. As indicated on page 5-4 of the draft, EPA considered those studies supportive of a point of departure to derive an RfV based on some of these strengths. EPA (2008, p. 4-137) indicates that reduced birth weight was found in five studies but does not discuss the consistent finding of tetrachloroethylene developmental toxicity at similar concen-

trations in Tinston (300 ppm), Carney et al. at (249 ppm), Schwetz et al. (300 ppm), and Szakmary et al. (221 ppm) or the conflicting finding of no developmental toxicity at 500 ppm in the Hardin et al. (1981) study. The limitations of Hardin et al. (single exposure level and lack of minimal maternal toxicity), Schwetz et al. (single exposure level), Nelson et al. (1979) (insufficient study details), and Szakmary et al. (lack of dose-response relationship because of excessive maternal toxicity at higher exposure levels) also are not discussed. In addition, the studies that do not conform to EPA and OECD regulatory testing guidelines are not identified.

STRENGTH OF EVIDENCE

The summary of the data on the developmental toxicity of tetrachloroethylene from selected studies is not particularly helpful, because EPA did not present its evaluation of the information and the basis for citing particular studies and study results is unclear. For example, EPA cites limited developmental-toxicity studies, such as Szakmary et al. (1997) and Schwetz et al. (1975), but does not cite Carney et al. (2006), the strongest one. EPA's reason for citing tetrachloroethylene-induced behavioral changes as evidence of developmental toxicity in the summary also is not clear, and the citation does not seem to be supported by the data. Tetrachloroethylene's effects at 1,000 ppm in the Tinston (1994) study are described on page 4-131 as central nervous system (CNS) depression and in Table 4-9 as behavioral effects. CNS depression appears to be more accurate on the basis of the symptoms described. The behavioral effects reported by Szakmary et al. (1997) are confounded by excessive maternal toxicity, and tetrachloroethylene had minimal effects on the behavior of rats in the study by Nelson et al. (1979). EPA provides no summary information on the reproductive toxicity of tetrachloroethylene even though data are available from a well-conducted two-generation reproduction study (Tinston 1994). Stating whether tetrachloroethylene can be identified as a developmental toxicant or a reproductive toxicant according to the criteria in the EPA developmental-toxicity risk-assessment guidelines (EPA 1991) and reproductive-toxicity risk-assessment guidelines (EPA 1996) would be helpful to risk managers and others and would help to identify data gaps.

For example, *there is sufficient evidence to identify tetrachloroethylene as a developmental toxicant in experimental animals* on the basis of the results of Carney et al. (2006) and Tinston (1994). That conclusion is consistent with the developmental-toxicity risk-assessment guidelines (EPA 1996, p. 40), which state: "The minimum evidence necessary to judge that a potential hazard exists generally would be data demonstrating an adverse developmental effect in a single, appropriate, well-conducted study in a single experimental animal species." *There is insufficient evidence to indicate that tetrachloroethylene does not cause reproductive toxicity in experimental animals* on the basis of the negative findings on reproductive performance and fertility in Tinston. That conclusion is

consistent with the reproductive-toxicity risk-assessment guidelines (EPA 1991, p. 72), which state: "The minimum evidence needed to determine that a potential hazard does not exist would include data on an adequate array of endpoints from more than one study with two species that showed no adverse reproductive effects at doses that were minimally toxic in terms of inducing an adverse effect. Information on pharmacokinetics, mechanisms, or known properties of the chemical class may also strengthen the evidence."

ATTRIBUTING DEVELOPMENTAL TOXICITY TO TRICHLOROACETIC ACID

EPA's speculation in Section 4.7.4 of the draft that trichloroacetic acid (TCA) is the causative agent in the developmental toxicity of tetrachloroethylene does not seem scientifically sound, and the discussion is not balanced. The available scientific data appear to contradict EPA's speculation. In the studies by Schwetz et al. (1975) and Carney et al. (2006), trichloroethylene (in contrast with tetrachloroethylene) did not cause developmental toxicity even though higher concentrations of TCA should have been produced from trichloroethylene than from tetrachloroethylene. In addition, tetrachloroethylene and TCA produce different types of developmental toxicity. Oral administration of TCA has consistently produced cardiac malformations in rats (Smith et al. 1989; Johnson et al. 1998). Dichloroacetic acid (DCA) also produces cardiac malformations when administered orally to rats (Smith et al. 1992; Epstein et al. 1992). The malformations produced by TCA and DCA are consistent with the teratogenic potential of other weak acids, such as valproic acid and ethylhexanoic acid (Scott et al. 1994), but are not consistent with tetrachloroethylene-induced developmental toxicity. The developmental toxicity produced by tetrachloroethylene did not include cardiac malformations in any of the studies described by EPA in Section 4.7.2. EPA's discussion of the evidence supporting TCA as the causative agent in tetrachloroethylene developmental toxicity is not balanced. EPA did not comment on the relatively high concentrations of TCA required to cause developmental toxicity compared with the concentration expected to result from metabolism of tetrachloroethylene in vivo or on whether this could account for the difference in the type of developmental effects that result from tetrachloroethylene exposure. The lack of information on the availability of metabolized TCA to the developing fetus and the potential differences related to oral vs inhalation exposure in the TCA and tetrachloroethylene studies, respectively, also were not addressed.

EPIDEMIOLOGIC STUDIES

Few epidemiologic studies bear on possible associations between exposure to tetrachloroethylene and the specific adverse reproductive outcomes considered. Most of the available studies have serious methodologic limitations and so

are not particularly informative as to the potential adverse reproductive effects of tetrachloroethylene exposure. Challenges that commonly confront investigators conducting epidemiologic studies of environmental determinants of reproductive health were evident in the available literature, specifically, standard case definitions, systematic ascertainment of end points, correct classification of exposure with respect to timing of pregnancy, and specificity of exposure to tetrachloroethylene.

The draft IRIS assessment considered the evidence on reproductive effects of tetrachloroethylene to be limited but cited spontaneous abortion as the outcome for which the evidence of an association with tetrachloroethylene was strongest on the basis of results in three papers (Kyyronen et al. 1989; Olsen et al. 1990; Doyle et al. 1997). In general, the committee agrees with EPA's assessement but takes a cautious view of inferences about the reproductive effects of tetrachloroethylene. The committee considered the work by Doyle et al. (1997) and Kyyronen et al. (1989) to be the most methodologically sound because they were based on cohorts of employed women about whom there was some information on tetrachloroethylen exposure and there was adequate evidence that the spontaneous abortions were validly reported. The studies examined spontaneous abortion in recognized pregnancies in cohorts of dry-cleaning and laundry workers; both reported an increased risk of spontaneous abortion in women who worked in dry-cleaning while pregnant. Nevertheless, both studies were limited by potential selection bias and small sample sizes and did not adequately address early fetal loss. They provide limited but supportive evidence of an association between tetrachloroethylene exposure and spontaneous abortion. The other study that EPA found compelling was that by Olsen et al. (1990); this study, although methodologically sound, was limited by the small number of events in the exposed groups.

There was also limited evidence of effects of tetrachloroethylene exposure on the developing fetus in a well-designed study from Camp Lejeune, North Carolina (Sonnenfeld et al. 2001). An increase in small-for-gestational-age cases was observed in children born to older women and women who had a history of fetal loss, but little effect was observed in other segments of the population. That discrepancy was difficult to resolve and may be spurious. (After publication of this study, it was discovered that some members of the control population were misclassified and were actually exposed, so the analyses in the paper are no longer valid.) EPA is inconsistent in characterizing the strongest evidence of reproductive toxicity. In "Characterization of Hazard and Dose Response" (EPA 2008; Section 6.1.3, page 6-5, lines 5-6), EPA cites "some evidence for growth retardation in infants born to mothers residing in housing with drinking water contaminated with tetrachloroethylene" as the main evidence of a reproductive outcome of concern. That conflicts with the conclusions in Chapter 4, where EPA indicates that the strongest evidence is on spontaneous abortion on the basis of the occupational studies.

EPA also considered potential male-mediated effects of tetrachloroethylene (Eskenazi et al. 1991a,b). Semen-analysis measures in dry-cleaning and

laundry workers were compared. The reported differences were subtle and did not always favor the exposed or unexposed. The second study examined total fertility in the wives of dry-cleaners by using standardized fertility ratios; this study was uninformative in that it was too small to evaluate fertility patterns.

In general, the committee did not consider the draft section on adverse reproductive and developmental outcomes to be balanced in the presentation or critique of studies. The committee's general impression was that the section focused primarily on studies that reported results that confirmed a positive association and that the effect of methodologic limitations of the studies on the validity of results was not fully appreciated. For example, in discussing possible reasons for failure to find associations between tetrachloroethylene exposure and adverse outcomes (page 4-121, line 33, through page 4-122, line 7), the draft did not consider the possibility that there is no association. In another case, the draft assessment refers to a "strong but imprecise association between IUGR [intrauterine growth restriction] and exposure to tetrachloroethylene (OR =12.5, 95% CI not given" (page 4-122, lines 8-12), but this result is based on *a single exposed case*. EPA's description suggests an impressive finding. A more appropriate discussion would have stated there were too few exposed cases to calculate a measure of association reliably and would not have cited the odds ratio.

In addition, the draft includes some errors in reporting results. For example, the results of Windham et al. (1991; see page 4-120, lines 21-22) are reported to be adjusted for age, race, education, prior fetal loss, smoking, and number of hours worked, implying multivariable adjustment, whereas data were adjusted for these variables one at a time (see Windham et al. [1991], page 247, paragraph 3).

Finally, the discrepancy in emphasizing spontaneous abortion as the outcome with the strongest evidence of an association with tetrachloroethylene exposure in Chapter 4 and intrauterine growth retardation in Chapter 6 suggests that the evidence on reproductive outcomes was not carefully evaluated.

FINDINGS AND RECOMMENDATIONS

EPA's identification of the key animal and epidemiologic reproductive and developmental studies of tetrachloroethylene appears to be complete, but the committee recommends some reorganization and reconsideration of data to provide a more transparent and balanced characterization of the data. The committee agrees with the selection of the Tinston (1994) two-generation reproductive-toxicity study and the Carney et al. (2006) developmental-toxicity study as supportive of a point of departure and an RfV. EPA's derivation of a comparative RfV based on the developmental toxicity of tetrachloroethylene is an important contribution to the tetrachloroethylene database. However, the committee recommends that EPA revise the chapter to address the specific deficiencies discussed above regarding information presented on the animal reproductive and developmental studies. In particular, the revision should include: (1) a critical

analysis of the described studies, including an assessment of the relationship of maternal toxicity to developmental toxicity and the strengths, limitations, and consistency of the various study results; (2) characterization of maternal toxicity (e.g., mild or severe) associated with the studies listed in Table 4-10 and use of consistent nomenclature (ppm or mg/m^3) for listing tetrachloroethylene concentrations; (3) the scientific basis for selecting the Tinston (1994) and Carney et al. (2006) studies as supportive of an RfV; (4) the scientific rationale for selecting the Tinston (1994) study instead of the Carney et al. (2006) study for derivation of the comparative RfV; (5) information on the mode of action for tetrachloroethylene-induced developmental toxicity which addresses the apparent contradictions raised in the committee's review that TCA may be the causative agent; and (6) characterization of the evidence for tetrachloroethylene-induced reproductive and developmental toxicity in animals based on EPA risk assessment guidelines. Stating explicitly whether the animal evidence is sufficient or insufficient for these important end points will help risk managers and others to more readily identify and protect against potential adverse health effects. It will also help to identify data gaps in the tetrachloroethylene database. In addition to revising the chapter, the committee also recommends that EPA consider conducting a bench-mark dose analysis and deriving an RfV based on the Carney et al. (2006) study in addition to, or instead of, the Tinston (1994) study. This will address the potential confounding effects of maternal toxicity at the 1,000 ppm exposure level observed in the Tinston (1994) study.

5

Genotoxicity

Whether tetrachloroethylene and its metabolites are genotoxic (and if so at what doses) is an important consideration in evaluating potential modes of action for carcinogenic effects in the Environmental Protection Agency (EPA) draft Integrated Risk Information System (IRIS) assessment. The evidence on the genotoxicity of tetrachloroethylene is summarized in Section 4.3 of the draft assessment (EPA 2008). The committee found that the publications cited and discussed by EPA are relevant but that the summary does not reflect the entire knowledge base available on the topic and does not provide transparent means for assessing the genotoxicity of tetrachloroethylene itself or its metabolites. The draft IRIS assessment predominantly reports positive studies, whereas good studies that had negative results are not mentioned or in some cases are incorrectly described as having had positive results. The committee therefore recommends that a more balanced, transparent, and inclusive approach be used to consider the evidence. The sections below offer some specific guidance.

ORGANIZATION AND EVALUATION OF DATA

The draft IRIS assessment's consideration of genotoxicity lacks cohesive structure, and the organization of the data presentation should be revised. Specifically, the section should be subdivided into sections on tetrachloroethylene itself, its metabolites, and evidence of indirect genotoxicity. Each section should include a table that lists all primary publications, the results related to tetrachloroethylene in the assays that it was tested in, and comments regarding strengths or weaknesses of each dataset. How the studies were selected should be articulated. It would be helpful if the studies were organized according to the general test systems used; for example, data on nonmammalian systems, in vitro mammalian cells, intact animals, and humans should be delineated separately. A good example of such table may be found in recent monographs of the International Agency for Research on Cancer (IARC). The text that accompanies each table should provide an assessment of the quality of each study cited. At the end

of each section, an evaluation of the strength of the evidence of genotoxicity of a particular compound should be included by way of summarizing the totality of data available. Finally, there should be an integrative assessment, including species-specific kinetics and metabolism of tetrachloroethylene and of genotoxicity and mutagenicity in intact animals and humans.

STUDIES OF TETRACHLOROETHYLENE

Nonmammalian Systems

A considerable number of mutagenicity studies of pure tetrachloroethylene that used *Salmonella* strains, *Escherichia coli*, and *Saccharomyces* have been performed with and without exogenous metabolic activation by liver S9 fractions from rats, mice, and hamsters (including animals pretreated with Aroclor or phenobarbital). The results have been essentially negative. The studies should be documented in a table (see above for specific format suggestions). However, when tetrachloroethylene was incubated with purified glutathione *S*-transferase (GST), glutathione, and rat kidney fractions, formation of *S*-(1,2,2-trichlorovinyl) glutathione (TCVG) was found, and mutagenic activity in *Salmonella* was clearly demonstrated as correctly described in the EPA draft.

The committee recommends that EPA also consider the negative results in the National Toxicology Program study (NTP 1986) of sex-linked lethal mutations in *Drosophila*.

Mammalian Cells in Vitro

EPA should describe the mutation study with mouse lymphoma L5178Y cells (NTP 1986), which appears to be the only available mammalian mutation test performed with tetrachloroethylene. This well-done study revealed that tetrachloroethylene at a variety of concentrations, with and without S9 for metabolic activation (but not with GST and rat kidney fractions), did not enhance the frequency of mutations at the thymidine kinase locus. Likewise, investigations of chromosomal aberrations and sister-chromatid exchanges (SCEs) in Chinese hamster ovary (CHO) cells (NTP 1986; Galloway et al. 1987) showed no evidence of tetrachloroethylene-induced genetic activity, although for technical reasons the weight of these studies was somewhat limited. In addition, the negative studies of chromosome aberrations in Chinese hamster lung cells by Sofuni et al. (1985) should be reported.

The work of Hartmann and Speit (1995) is addressed in the draft IRIS assessment, but it is incorrectly quoted in a statement that tetrachloroethylene induced genetic damage, which was not shown. Hartmann and Speit investigated SCEs and DNA integrity (by using the single-cell gel electrophoresis or comet assay) in human blood cells exposed to tetrachloroethylene in vitro. The study was well performed, with negative and positive controls, without and with

metabolic activation, and with assay repeats. Although the highest concentration of tetrachloroethylene used in the comet assay was cytotoxic, there clearly was no evidence that tetrachloroethylene at any dose caused increases in SCEs or comet. EPA's review of the study should be corrected.

Concerning the study of Doherty et al. (1996), the EPA draft correctly reports that tetrachloroethylene induced micronuclei in two novel cell lines of human lymphoblastoma origin (h2E1 and MCL-5) through either clastogenic or aneugenic mechanisms. Cells were genetically engineered to express human enzymes (CYP2E1 or CYP1A2, 2A6, 3A4, 2E1) and epoxide hydrolase stably. The committee recommends that EPA acknowledge that those cell lines were not validated as test systems and that other compounds tested in the study, such as hexane and toluene, that are generally regarded as nongenotoxic also led to formation of micronuclei—an indication that the new cell lines may be oversensitive and may provide false-positive results. Micronucleus formation in MCL-5 cells by tetrachloroethylene was confirmed by White et al. (2001), and Wang et al. (2001) found increases in micronuclei in CHO-K1 cells, as mentioned in the draft IRIS assessment.

Tetrachloroethylene's effects on unscheduled DNA synthesis were studied in human fibroblasts (WI-38) (Beliles et al. 1980), in primary hepatocytes from rats and mice (Shimada et al. 1985; Costa and Ivanetich 1984; Milman et al. 1988), and in human lymphocytes (Perocco et al. 1983); the results were mostly negative. Although those studies are limited in performance or reporting, EPA should discuss them to provide a full account of the existing database.

In Vivo Studies in Animals

EPA correctly reports that the study of Walles (1986) showed occurrence of DNA single-strand breaks in liver and kidneys but not lungs of mice 1 hour after intraperitoneal injection of tetrachloroethylene at 650-1,300 mg/kg dissolved in 0.05 mL of Tween 80. EPA fails to mention the full reversibility of that effect at 24 hours. Furthermore, the relevance of the unphysiologic mode of application (intraperitoneal injection in Tween) should be discussed. Tetrachloroethylene is a known irritant of skin and mucosa, and intraperitoneal injection may trigger the release of inflammatory mediators that will stimulate secretion of reactive oxygen species and cytokines in liver and kidney. In addition, the high toxic dose of tetrachloroethylene may produce cell death associated with endonucleolytic DNA fragmentation (Storer et al. 1996). No increase in renal single-strand breaks in DNA was seen 24 hours after oral administration of tetrachloroethylene in rats, but single-strand breaks were enhanced after application of the genotoxins dimethylnitrosamine and diethylnitrosamine (Potter et al 1996).

The EPA draft quotes the paper by Mazullo et al. (1987), which reports low levels of DNA binding 22 hours after intraperitoneal injection of radioactively labeled tetrachloroethylene in mice or rats. Binding was calculated at 2.9

pmol/mg for mouse liver DNA and 0.2-0.5 pmol/mg for rat liver and rat and mouse kidney, lung, and stomach DNA. Thus, there was no evidence of increased binding to rat kidney DNA as misleadingly reported by EPA. Moreover, EPA fails to mention that RNA and protein were labeled much more highly than DNA (up to 420 pmol/mg in the case of RNA). That seriously limits the weight of the study because DNA may have been contaminated by RNA or protein (apparently, DNA was not purified to constant specific activity) and ^{14}C may have been incorporated into DNA via the intermediary metabolism. Overall, those limitations should be taken into account by EPA in the evaluation of the study.

The in vivo micronucleus study in mice by Murakami and Horikawa (1995) is potentially of key importance in the evaluation of tetrachloroethylene's effects on intact organisms. The authors investigated the appearance of micronucleated cells in peripheral blood and liver. However, the draft IRIS assessment is partially incorrect: it reports increased frequencies of micronuclei in peripheral blood reticulocytes after intraperitoneal injection of tetrachloroethylene, but the paper says the opposite (that is, there was no increase in micronuclei in reticulocytes). EPA correctly quotes from the paper in saying that hepatocytes showed small increases in micronuclei when mice received intraperitoneal injections of tetrachloroethylene at high doses 24 hours after partial hepatectomy but not when tetrachloroethylene was injected before partial hepatectomy. The frequency of micronuclei increased less than two-fold but was statistically significant; the positive control diethylnitrosamine produced a 10-fold increase. Several restrictions should be considered by EPA in interpreting the study. The effects were observed at high doses (1,000 and 2,000 mg/kg were effective, but not 500 mg/kg). Given that hepatic toxicity in mice increases from a lowest observed-adverse-effect level of 100 mg/kg (EPA 2008, Section 4.4.2.1), the high doses necessary to enhance micronucleus formation must have been severely toxic to the residual hepatocytes and to the whole organism. The toxic load on the residual liver would have been aggravated by the intraperitoneal tetrachloroethylene application and by the likely release of cytokines and reactive oxygen species. Overall, the small observed increase in micronuclei in mouse hepatocytes might have been due to nonspecific toxic effects. In conclusion, this in vivo study clearly found no increase in reticulocyte micronuclei, and the data suggesting formation of micronuclei in hepatocytes are not convincing.

EPA should mention the in vivo unscheduled DNA synthesis test performed on kidney. Tetrachloroethylene was administered to rats orally (1 g/kg at 0 and 12 hours); at 24 hours, no evidence of unscheduled DNA synthesis in isolated renal cells was observed (Goldsworthy et al. 1988, abstract).

A recent paper by Cederberg et al. (2009) describes the results of an in vivo study in which the alkaline Comet assay was performed on the liver and kidney of CD1 mice treated orally with tetrachloroethylene at 1,000 or 2,000 mg/kg dissolved in corn oil. A slight increase in DNA damage was reported; the effect was significant for one of two end points (tail intensity, but not tail moment) in the liver. No increases were found in the kidney. The study had been performed by a contract laboratory, and the study director had concluded from

the same data that tetrachloroethylene did not increase DNA damage because of the inconsistent effects on the two end points, the low magnitude of increases, the high inter-animal variation, and lack of statistically significant increases in a statistical test (Dunnet). Overall, the paper by Cederberg et al. does not present convincing evidence for a genotoxic activity of tetrachloroethylene.

It would also be useful to add the results of studies of hepatic-tumor initiation by tetrachloroethylene although this end point does not necessarily reflect mutagenic activity. When 10 male Osborne Mendel rats were given tetrachloroethylene at 1,000 mg/kg and then phenobarbital as a promoting treatment for 7 weeks (an initiation protocol), the tetrachloroethylene did not induce an increase in the number of gamma-glutamyl transpeptidase-positive cell foci in the liver (Milman et al 1988). Likewise, tetrachloroethylene did not produce liver foci in neonatal female Wistar rats exposed at 2,000 ppm 8 hours/day 5 days/week for 10 weeks (Bolt et al. 1982). Thus, two independent studies did not indicate an initiation potential of tetrachloroethylene in rat liver.

Studies in Humans

Toraason et al. (2003) studied oxidative damage (measured as 8-hydroxydeoxyguanosine [8-OHdG]) in leukocyte DNA of 18 female dry cleaners exposed to tetrachloroethylene and compared it with oxidative damage in 20 female laundry workers who were not exposed to tetrachloroethylene. Blood concentrations in the exposed workers were greater than in unexposed workers by two orders of magnitude. There was a statistically significant reduction in 8-OHdG in the exposed workers and no difference in urinary 8-OHdG or in a urinary lipid peroxidation biomarkers between the two groups. The data from this small sample provide no evidence of oxidative DNA damage under the conditions of the study.

EPA should report the studies by Ikeda et al. (1980a,b), who investigated chromosomal aberrations, SCEs, and modified cell-cycle kinetics in human lymphocytes after 3 days in culture with phytohemagglutinin. Lymphocytes were obtained from 10 workers who had been exposed to tetrachloroethylene and from 11 control subjects. Although no significant effects were found in the exposed group with respect to any of the end points, the limitations of the studies, such as small samples, will need to be considered in evaluating the results.

STUDIES OF METABOLITES OF TETRACHLOROETHYLENE

EPA briefly describes studies that identify TCVG, *S*-(1,2,2-trichlorovinyl)-L-cysteine (TCVC), and *N*-acetyl-*S*-(1,2,2-trichlorovinyl)-L-cysteine (*N*-Ac-TCVC) as bacterial mutagens that act either directly or after activation by rat renal microsomes. It also mentions the induction of unscheduled DNA synthesis by TCVC in a porcine renal-cell line and the key role of renal ß-lyase in the final activation step as demonstrated in these studies.

The EPA draft mentions the positive test for bacterial mutagenicity of tetrachloroethylene epoxide. A discussion of existing studies of the genotoxicity of trichloroacetyl chloride should be added. As to trichloroacetic acid (TCA), the draft states (EPA 2008, p. 4-5) that "as reviewed by Moore and Harrington-Brock (2000), the oxidative metabolite TCA, the major urinary excretion product, exhibits little, if any, genotoxic activity." That statement is followed by brief descriptions of numerous studies of single-strand breaks, which had inconsistent results. Increases in single-strand breaks might have been caused by cytotoxic effects and necrosis at high doses of TCA because of endonucleolytic degradation of DNA (Storer et al. [1996], as reported by EPA). The purpose of the description of studies devoted exclusively to DNA single-strand breaks after exposure to TCA is not clear. The committee recommends integration of the data on single-strand breaks into a balanced review of all available genotoxicity studies of TCA (including a table and a discussion of the studies' strengths and weaknesses) to support the conclusion that TCA exhibits little if any evidence of genotoxicity by an evaluation of the weight of evidence.

Clarity regarding the genotoxicity studies of chloral hydrate and dichloroacetic acid (DCA) is also needed. As recommended earlier, this would be facilitated by an overview of all published data displayed in tables, and there should be a weight-of-evidence evaluation to support EPA's conclusion that chloral hydrate and DCA are genotoxic. That conclusion generally agrees with a recent IARC assessment, but according to IARC (2004), genotoxicity of DCA was limited to high doses that probably are not relevant to tetrachloroethylene carcinogenicity; EPA should consider this argument.

TCVC sulfoxide, another reactive metabolite of tetrachloroethylene, which is nephrotoxic (Elfarra and Krause 2007), does not appear to have been studied for genotoxicity.

EVIDENCE OF INDIRECT GENOTOXICITY

Two studies by Toraason et al. (1999, 2003) are briefly described in the draft IRIS assessment. They revealed no evidence of oxidative DNA damage in rats after a single intraperitoneal dose of tetrachloroethylene at up to 1,000 mg/kg in rats or in humans after occupational exposure to tetrachloroethylene. EPA should add the important information from the animal study by Toraason et al. (1999) that the similar chemical trichloroethylene applied at the same doses as tetrachloroethylene increased oxidative DNA damage in rat liver, whereas tetrachloroethylene did not.

As reported in the IARC (2004) monograph on TCA, the frequency of 8-hydroxydeoxyguanosine-DNA adducts in the liver of $B6C3F_1$ mice was not modified after application of TCA via drinking water (Parrish et al. 1996), was slightly increased after administration through gavage (Austin et al. 1996), and was clearly increased after intraperitoneal injection (Von Tungeln et al. 2002). That comparison of study results again suggests that the route of application

(oral vs intraperitoneal) should be considered in evaluating genotoxic effects of tetrachloroethylene and its metabolites.

FORMATION OF REACTIVE METABOLITES IN ANIMALS AND HUMANS

As described in Section 3 of the draft IRIS assessment, the metabolic flux of tetrachloroethylene through glutathione conjugation and β-lyase cleavage is much lower in humans than in rats. TCVG formation in liver, β-lyase activity in kidney, and *N*-Ac-TCVC excretion in urine are all much lower in humans than in rats (Dekant et al. 1986b; Green et al. 1990; Volkel et al 1998). Furthermore, Pahler et al. (1998, 1999) generated monospecific antibodies to the protein adducts of the reactive intermediates either of the glutathione (GSH) conjugation or the oxidative pathway, namely to *N*-dichloroacetyl-L-lysine and *N*-trichloroacetyl-L-lysine. The antibodies allow determination of the amounts of reactive metabolites formed in the two main pathways. Comparing binding in rat kidney and rat liver subfractions, the dichloro adduct (indicating the GSH conjugation pathway) predominates in the kidney with only faint bands in liver; the trichloro adduct (indicating the oxidative pathway) predominates in the liver. Pahler et al. (1999) also compared protein adducts in rat plasma and human plasma obtained from six volunteers. Both adducts were present in rat plasma; in human plasma, the dichloro adducts were below the detection limit, and the trichloro adduct was much lower than in rat plasma. It can be calculated from the data that after exposure to tetrachloroethylene at the same concentration (40 ppm) and duration (6 hours), dichloro adducts were at least 40-fold lower in human plasma than in rat plasma. Trichloro adducts were not quantifiable with gas chromatography for technical reasons (Pahler et al. 1999).

Overall, those results show that humans produce smaller amounts of the reactive metabolites; this is consistent with the overall greater metabolism of tetrachloroethylene in rats. A possible risk of mutagenic effects posed by tetrachloroethylene metabolites with known genotoxic activity should therefore be substantially lower in humans than in rats. However, not all possible metabolites have been assessed for mutagenic activity, and techniques for identifying some metabolites in human samples are not readily available.

Generally, the committee recommends that EPA integrate the qualitative and quantitative data from toxicokinetic, metabolic, and toxicodynamic studies in its assessment of the current knowledge of the toxic potential of tetrachloroethylene and specifically in its mode-of-action considerations.

CELL-TRANSFORMATION ASSAYS

The committee recommends that EPA include at least the more recent cell-transformation studies of tetrachloroethylene (Tu et al 1985, Milman et al. 1988).

FINDINGS AND RECOMMENDATIONS

In vitro studies did not provide evidence of mutagenic activity of tetrachloroethylene in mouse lymphoma cells or in bacterial and yeast mutation assays except in the few tests in which metabolites of the GSH pathway were generated, and no increases in chromosomal aberrations and SCEs were found in CHO cells. Tetrachloroethylene did not increase SCE and comet formation in human blood cells (this was incorrectly reported in the EPA draft); increases in the frequency of micronuclei were found in genetically altered human lymphoid cell lines and in a CHO cell line. In vitro studies of unscheduled DNA synthesis were mostly negative.

The key question is whether the reactive metabolites of tetrachloroethylene are formed and become available to sensitive cells in vivo and have genotoxic effects in intact organisms. Tetrachloroethylene did not induce unscheduled DNA synthesis in rat kidney. It induced single-strand breaks in mouse liver and kidney at 1 hour but not at 24 hours after intraperitoneal injection and not in rat kidney 1 day after oral administration. The increase at 1 hour may be nonspecific because of intraperitoneal application and high doses. Tetrachloroethylene did not increase micronucleated reticulocytes in peripheral blood of mice (this was incorrectly reported in the EPA draft) and did not increase micronucleated hepatocytes when administered before partial hepatectomy. When injected after partial hepatectomy, tetrachloroethylene slightly increased micronucleus formation, but this effect may be nonspecific because of severe liver toxicity caused by the high doses of tetrachloroethylene and the intraperitoneal application of this irritant substance. A study with ^{14}C-labeled tetrachloroethylene suggested a low level of binding to mouse liver DNA and even less to rat liver DNA and mouse and rat kidney, lung, and stomach DNA. These effects are considered nonspecific because DNA was not purified to constant radioactivity and because labeling via the intermediary metabolism appeared likely. In humans exposed to tetrachloroethylene, no evidence of genetic alterations was noted, although the studies are of limited weight. Two studies in rats found no evidence of tumor-initiating activity of tetrachloroethylene (when liver foci were used as the end point).

In conclusion, there is no convincing evidence that tetrachloroethylene has important genotoxic or mutagenic activity in intact organisms. The committee agrees with EPA's conclusion that several metabolites of tetrachloroethylene are clearly genotoxic: TCVG, TCVC, *N*-Ac-TCVC, tetrachloroethylene oxide, DCA, and chloral hydrate. However, it is still questionable whether the metabolites of tetrachloroethylene play an important role in the mode of action of tetrachloroethylene carcinogenesis (see Chapters 6-8) in view of the absence of convincing evidence of mutagenic and tumor-initiating activity of tetrachloroethylene in vivo. Additional studies of genotoxicity in vivo with state-of-the-art methods would be valuable.

As noted above, the committee recommends that EPA provide an expanded and more integrated discussion of the genotoxicity data. The presentation could be improved by the use of tables detailing the primary evidence, by

separate discussion of the genotoxic evidence on tetrachloroethylene and its metabolites, and by a more critical analysis of the studies.

6

Hepatic Toxicity and Cancer

This chapter reviews information presented in the Environmental Protection Agency (EPA) draft Integrated Risk Information System (IRIS) assessment of the toxic and carcinogenic effects of tetrachloroethylene on the liver. The metabolism of tetrachloroethylene by the liver is critical for its toxicity and carcinogenicity in that organ. The major metabolites of tetrachloroethylene responsible for hepatic effects are formed by the oxidative metabolic pathway (see Chapter 2 for an overview of toxicokinetics). The following sections address hepatotoxicity and hepatocarcinogenicity separately, but they are not necessarily independent end points. This information is considered in the context of the other evidence on carcinogenicity in Chapter 11, where EPA's assessment of carcinogenic risks posed by tetrachloroethylene is evaluated.

HEPATOTOXICITY

Animal Studies

The draft IRIS document on tetrachloroethylene points out that hepatotoxicity associated with tetrachloroethylene has been shown in rodents in several studies. A number of studies have been conducted with acute administration, but the draft correctly focuses on subchronic and chronic exposures, particularly those involving inhalation as a route of administration. Most of the toxicologic findings focus on increased liver weight, hypertrophy, and histologic lesions, including necrosis.

Damage to the liver by all or most of the chlorinated hydrocarbons has been demonstrated. Tetrachloroethylene is a weaker hepatotoxic agent than, for example, carbon tetrachloride and chloroform; this was shown by studies conducted in the middle 1960s (Klaassen and Plaa 1966, 1967).

The IRIS document overemphasizes a few studies. One is that by Kjellstrand et al. (1984), which is also mentioned in Chapter 3, on neurotoxicity. According to that study, exposure to tetrachloroethylene at 9 ppm for 30 days

caused a significant increase in liver weight (not corrected for body weight) in mice. The study also reported an increase in plasma butyrylcholinesterase (BuChE) in mice exposed to tetrachloroethylene at over 9 ppm for 30 days. Although the importance of the change in BuChE is not clear, the exposure in the study was so much lower than those in the other studies cited by EPA that it is important in considering the noncarcinogenic liver end points. EPA does not note that the increase in BuChE at 9 ppm was not significant (it was significant only at 37 ppm and above). It would be valuable for EPA to discuss this study critically in comparison with others in which much higher lowest observed-adverse-effect levels were found. In particular, it should be mentioned that increased BuChE in the Kjellstrand et al. study occurred at 37 ppm only when the exposure was continuous for the entire period, not when exposure at this concentration was intermittent, whereas other studies have involved intermittent exposure (usually 3-6 hours/day). Therefore, the total dose per mouse in the Kjellstrand et al. study must have been several times higher than that in other studies, and the information given in the draft (p. 4-12 and Table 4-2 on p. 4-14) is misleading. It would also be useful for EPA to discuss the quality of studies (for example, deficiencies in reporting by Kjellstrand et al.) and the toxicologic meaning, if any, of the reported effects. Furthermore, the increase in BuChE as a toxic effect does not appear to have been considered important by other investigators, on the basis of citations of the Kjellstrand et al. paper, nor does the effect seem to have been reported by others. Thus, a more critical analysis of the study is necessary to determine the significance of its findings in comparison with other reports of hepatotoxicity that required higher exposure concentrations.

The National Toxicology Program (NTP 1986) and Japan Industrial Safety Association (JISA 1993) studies lend some support to the possibility of hepatotoxicity associated with exposure to tetrachloroethylene. In the NTP 13-week study in rats, hepatotoxicity was evidenced as congestion in the liver. In the 13-week study in mice, there was leukocytic infiltration, centrolobular necrosis, and bile stasis in animals exposed to tetrachloroethylene at 400, 800, or 1,600 ppm. Liver degeneration was observed to occur in a dose-dependent fashion in the 2-year study in mice. In the JISA study, there was an increase in spongiosis hepatitis in Crj:BDF_1 mice, but it is a common finding in these mice and is likely to be unrelated to chemical exposure. Hyperplasia was not statistically significantly increased; there were increases in angiectasis and central degeneration.

In updating and revising the draft IRIS assessment, EPA should include a new 30-day gavage study in Swiss Webster mice given tetrachloroethylene at 150, 500, and 1,000 mg/kg/day (Philip et al. 2007). The metabolism of tetrachloroethylene and its toxicity were examined. That is one of the few studies that were conducted with oral administration and repeated dosing. The investigators found that hepatic injury peaked at 7 days but then was repaired. That suggests that single-dose studies demonstrating hepatic damage on the basis of measurements made after short periods might not mimic the effects of repeated dosing.

Human Studies

EPA also discusses hepatotoxicity in humans. Most of the studies cited in the IRIS draft involved dry-cleaners and found no evidence of an association. However, the EPA document gives undue weight to a couple of studies. One (Brodkin et al. 1995) used sonographic analysis of scattering of fat in liver. This was the only study to report such effects in tetrachloroethylene exposed populations and the importance of the fat changes as an indicator of toxic response is unclear. Furthermore, serum transaminases were not increased in the exposed population. Thus, interpretation of the result is difficult. EPA also considers the study of Gennari et al. (1992). They reported an increase in gamma-glutamyltransferase-2 in tetrachloroethylene-exposed dry-cleaners. The relevance of that finding as an indicator of hepatotoxicity is unclear. The investigators did not find any other indicators of hepatotoxicity despite an extensive serum-enzyme profile. It is likely that the concentrations of tetrachloroethylene that humans were exposed to in those studies were too low to induce frank hepatotoxicity. Further studies are needed.

HEPATOCARCINOGENICITY

Animal Studies

The NTP (1986) and JISA (1993) studies showed, as is the case with many of the halogenated solvents, that there is a dose-dependent increase in hepatic tumors after exposure to tetrachloroethylene in both sexes of mice but not in rats. The draft IRIS assessment's section on hepatic carcinogenicity is written reasonably well in a descriptive sense, with regard to the style of the presentation of the cancer-relevant results of long-term studies with tetrachloroethylene. However, the presentation would benefit if the table on page 5-37, which now gives cumulative tumor incidence, were expanded to include information on species; strain; dose; duration; incidence and multiplicity of adenomas, carcinomas, and other hepatic tumors (such as hemangiosarcomas); and the literature cited.

Tetrachloroethylene induces hepatocellular carcinomas and adenomas in mice. The yield of tetrachloroethylene-induced hepatocellular carcinomas is statistically significant in both male and female $B6C3F_1$ mice after either oral or inhalation exposure. Both male and female $Crj:DBF_1$ mice also have an increased incidence of hepatocellular carcinomas after inhalation exposure to tetrachloroethylene. The earlier studies of the National Cancer Institute (NCI 1977) were repeated, and the findings were confirmed by Nagano et al. (1998). As discussed in more detail below in the section on mode of action, some metabolites of tetrachloroethylene—including trichloroacetic acid (TCA), dichloroacetic acid (DCA), and chloral hydrate (if it is formed)—cause hepatic cancer in mice, and DCA causes hepatic cancer in rats. In the study by Nagano et al., both

males and females incurred dose-related increases in incidences of hepatic carcinoma and combined hepatic adenoma and carcinoma.

A difficulty in interpreting the significance of the mouse hepatic tumors is that they have a high spontaneous background incidence in mice. Such tumors have been commonly encountered after exposure to other halogenated solvents, such as dichloromethane, trichloroethylene, tetrachloroethane, carbon tetrachloride, and 1,1,2-trichloroethane.

The curious observation of hepatic and splenic hemangiosarcomas reported in male mice in one of the tetrachloroethylene mouse bioassays (JISA 1993) is mentioned several times in the EPA draft as a potentially important finding; however, there is little discussion of these tumors, the potential mode of action, or the relevance to human risk. Reference to the tumors is presented in Figure 5-14, Table 5-5, and Table 5-9. The analysis is complicated by the fact that the JISA report does not describe the tumors as hemangiosarcomas, but rather as hemangioendothelioma; this term is usually associated with benign tumors, but JISA lists it as a malignant hepatic tumor in male mice. The term is also used for both benign and malignant tumors of the spleen. Furthermore, because of the cell types involved, the hepatocellular carcinomas being of hepatocellular origin and the hemangiosarcomas being of endothelial-cell origin, it is scientifically inappropriate to lump these tumors in with carcinomas, as is done by EPA (Figure 6.4 and Table 6.4).

Human Studies

Available epidemiologic evidence does not support an association between tetrachloroethylene and hepatic cancer. Two cohort mortality studies of dry-cleaner union members (Ruder et al. 2001; Blair et al. 2003) and a large (N = 77,965) cohort mortality study of aerospace workers (Boice et al. 1999) report no association with hepatic-cancer mortality. A sizable subcohort (N = 2,631) of the aerospace workers routinely exposed to tetrachloroethylene had a standardized mortality ratio of 2.05 (95% confidence interval [CI], 0.83-4.23) on the basis of seven observed deaths. However, an analysis that used an internal cohort referent population to reduce confounding yielded no overall association and no exposure-response relationship. Because hepatic cancer is fatal, assessments of mortality represent the burden of the disease in the population. Essentially null associations are reported in studies of incident cancers in laundry workers residing in Nordic countries. In the one study cited (Lynge et al. 1995) that reported an increased standardized incidence ratio (SIR) for hepatic cancer in women (2.7; 95% CI, 1.5-4.5; 14 observed cases, all cases were in laundry workers, and no cases were observed in dry-cleaning workers, whose exposure to tetrachloroethylene is more likely. (The EPA document does not cite this correctly in Table 4B-1a; the reference should be to Lynge et al. 1995, which is an update of Lynge and Thygesen 1990.) Those studies identified laundry and dry-cleaning workers on the basis of the census in 1970 and 1980, so the extent of

exposure is unknown. Several population-based case-control studies of hepatic cancer and exposure to solvents (determined by occupation) have been conducted over the last 30 years. Overall, they have not reported an association between tetrachloroethylene and hepatic cancer. Some evidence is suggestive of an association between solvent exposure and laundry work and hepatic cancer in women, but the exposure models for these studies are crude, and methods of control selection raise questions about the validity of the results.

The draft IRIS assessment does not use that limited evidence of an association between tetrachloroethylene and hepatic cancer as supportive of classifying tetrachloroethylene as a carcinogen. The argument that human epidemiologic evidence supports classification as "likely to be a carcinogen" is limited to other cancers, specifically esophageal and lymphoid cancers. The exclusion of hepatic cancer as supporting evidence is appropriate.

Mode of Action

The draft assessment describes the mode of action (MOA) of tetrachloroethylene's hepatic toxicity and carcinogenicity in several places. The most comprehensive description of the available body of information and identification of potential key events in the MOA are included in Section 4.4.4. The MOA summary is provided in Section 4.10.3, including Table 4-13; Appendix 4A details the EPA-conducted analysis of the consistency between carcinogenicity of tetrachloroethylene and that of one of its major oxidative metabolites, TCA; and Section 6.1.5 includes a short summary of the liver MOA with regard to the human hazard potential of tetrachloroethylene.

EPA concludes that "the MOA for tetrachloroethylene-induced mouse liver cancer is not well understood, and it is highly likely that more than one MOA is operative" (EPA 2008, p. 4-16). In support of that conclusion, EPA describes pathways that could lead to hepatic tumors but does not clearly describe the weight-of-evidence approach for determining the key elements in the tumorigenicity of tetrachloroethylene for the possible MOAs presented. The difficulty in characterizing the MOA is not surprising given the complexity of the metabolic pathways for tetrachloroethylene, the closely related chlorinated solvent trichloroethylene, and their common primary oxidative metabolites, TCA and DCA. The following major events are put forth as plausible components of the MOA of hepatic carcinogenicity of tetrachloroethylene (in no particular order with regard to a temporal sequence):

- Metabolism of tetrachloroethylene to TCA and DCA, which are both considered ultimate hepatotoxic metabolites.
- Activation of peroxisome proliferator-activated receptor-α (PPARα) and the downstream cascade of the molecular events that include induction of peroxisomes, increase in cell proliferation, and decrease in rates of apoptosis.

- Other nongenotoxic events, such as promotion of growth of previously initiated foci, changes in epigenetic status, cytotoxicity, and oxidative stress.
- Genotoxic events, such as DNA damage by tetrachloroethylene metabolites or chromosomal aberrations.

Although the discussion of the PPARα-mediated events and their possible roles in species differences with regard to the hepatocarcinogenic potency of tetrachloroethylene is extensive, other important potential MOAs or key events are largely overlooked. For example, the possible role of epigenetic changes caused by TCA and DCA is mentioned, but there is little discussion of the studies that have been conducted on this subject. Similarly, cytotoxicity and secondary oxidative stress that may result from microsomal enzyme induction are insufficiently considered. Adding such discussions would strengthen EPA's MOA analysis and conclusions.

That TCA is the major urinary metabolite of tetrachloroethylene and is a mouse hepatocarcinogen suggests that the hepatocarcinogenicity of tetrachloroethylene is due in part to TCA. DCA is another tetrachloroethylene urinary metabolite that is formed both in the oxidative pathway by dechlorination of TCA and, in organs other than the liver, in the glutathione (GSH) pathway. DCA is known to cause hepatic cancer in both rats and mice, so it is possible that DCA contributes to the hepatocarcinogenicity of tetrachloroethylene, although it is not certain to what extent it contributes in that little of it is produced and it is produced primarily in the kidney. Early studies that reported finding DCA as a metabolite may have overstated the amount formed because of problems with analytic methods (Ketcha et al. 1996). Later studies showed very small amounts of DCA, if any, being formed from tetrachloroethylene. Chloral hydrate (if it is formed) is a mutagen and is a hepatocarcinogen in mice and might contribute to the hepatocarcinogenicity of tetrachloroethylene. In addition, metabolites formed from the GSH pathway, such as trichlorovinylglutathione, which is further metabolized by β-lyase in the kidneys, are also genotoxic.

The multiplicity of metabolites formed from tetrachloroethylene that are toxic and carcinogenic—TCA, DCA, tetrachloroethylene oxide, trichloroacetyl chloride, and possibly chloral hydrate—makes it difficult to determine the MOA of hepatocarcinogenicity of tetrachloroethylene. Indeed, there may not be enough data to determine quantitatively the extent to which each metabolite contributes to tetrachloroethylene-induced hepatotoxicity. Perhaps a summary of the available information on hepatocarcinogenicity of TCA, DCA, and chloral hydrate administered alone or in combination with other compounds—for example, from studies of Bull et al. (1990, 2002, 2004) on mixtures and coadministration with gadolinium chloride—should be included in the IRIS assessment and in tabular form (e.g., see table in NRC 2006a, pages 149-156) to better assess the data.

Although the consideration of the metabolic activation of tetrachloroethylene and the comparison with TCA-induced carcinogenesis are useful, the dose-

response information in the draft on tumor formation after TCA administration (Table 4A-2) suggest that very high concentrations of TCA are needed to cause hepatic tumors—far beyond what would be generated after tetrachloroethylene administration.

The peroxisome-proliferator MOA is discussed in great detail. The key events associated with the known links between peroxisome-proliferator chemicals in general and rodent hepatic cancer are identified, and appropriate literature references are included. However, no data or weight of evidence criteria specifically on tetrachloroethylene are provided, and the lack of coherent flow in the document detracts from the intended message. The document might be improved by organizing the information into sections that make clear (1) what parts of this MOA are based on studies with other model peroxisome proliferators, (2) what data are available to support this MOA for tetrachloroethylene, (3) for TCA, (4) the rationale for species differences, and (5) the relevance of this MOA to mouse hepatic tumors induced by tetrachloroethylene or to human risk.

As presented, the draft IRIS assessment seems to be more concerned with critiquing the current dominant view in the field that the peroxisome-proliferator MOA may not be relevant to human hepatocarcinogenesis than with providing evidence of links between tetrachloroethylene and this MOA. The general criticism of the MOA with regard to its relevance to humans is warranted, although it should be expressed in milder terms, and it points correctly to several historical and recent lines of evidence that suggest important inconsistencies that challenge the paradigm of the central role of PPARα in rodent, but not human, hepatocarcinogenesis. However, as pointed out above, the data linking tetrachloroethylene to this MOA are weak to begin with and come largely from studies of trichloroethylene and TCA, not tetrachloroethylene itself. The idea that there are deficiencies in our knowledge of tetrachloroethylene should be made more prominent. Similarly, the discussion of "tetrachloroethylene and PPARα MOA" and the discussion of "relevance of the PPARα MOA to human liver carcinogenesis" should be separated more clearly by EPA.

The discussion of the strain and species differences in the peroxisome-proliferation effect of TCA is rather limited. TCA is capable of inducing peroxisome proliferation in the rat, but tetrachloroethylene does not. In addition, the issues of PPARα transactivation by tetrachloroethylene, related chemicals, and their key metabolites and of species differences are important for the discussion of the MOA. Again, a critical look at the quantitative differences in metabolic activation of tetrachloroethylene to TCA between mouse and rat, species that are generally believed to be almost equally sensitive to peroxisome proliferation and differences between mouse and rat in hepatic cancer induced by other compounds of this class should be provided. Specifically, EPA may consider performing additional analyses with the rat data similar to those with the mouse data in Appendix 4A and including a table showing the quantitative differences in affinity between mouse, rat, and human PPARα of tetrachloroethylene and its key metabolites in comparison with the known peroxisome proliferators. Such

analyses and data would greatly facilitate the discussion of quantitative differences between compounds and between species.

The study by Nakajima et al. (2000) is only mentioned in passing on page 4-26 of the draft assessment. It should be discussed in greater detail, especially the data on sex differences and mechanistic considerations. It provides a possible mechanistic explanation for sex differences in susceptibility to carcinogenesis by tetrachloroethylene—information that is important for the discussion of the complexities of and uncertainties in the MOA.

The dose-response relationship in Section 4.4.4.3.6 touches on the important issue. However, the arguments are not supported by adequate literature citations, and the only paper cited is a broad review article, not a primary source of the data. Section 5 contains ample information on dose-response relationships, so appropriate cross-referencing should be included in Section 4.4.4.3.6.

The discussion on nonliver targets in humans that may involve PPARα MOA is interesting, but it is too brief and is not adequately linked to the rest of the chapter to have an appropriate impact. The arguments presented in Section 4.4.4.3.8 may be substantiated by providing a quantitative comparison of PPARα transactivation potential by tetrachloroethylene and its metabolites, as suggested above. Similarly, the discussion of the potential role of PPARγ is inadequate. Specifically, it should be noted that PPARγ may be an important gene for human hepatocellular carcinogenesis.

The committee agrees with EPA that the MOA of tetrachloroethylene-induced hepatic tumors is not clear. Many toxic metabolites are formed from tetrachloroethylene. Hence, it is likely that key events from several pathways operate in tetrachloroethylene-induced hepatocarcinogenesis. It is likely that TCA, DCA, and chloral hydrate (if it is formed)—which are carcinogens in rodents—contribute to tetrachloroethylene-induced hepatocarcinogenesis. It is also likely that mutagenic metabolites of tetrachloroethylene formed via the cytochrome P450 and GSH pathways (tetrachloroethylene-epoxide, TCA, DCA, and TCVG) contribute to hepatocarcinogenesis. And it is possible that activation of PPARα and consequent peroxisomal proliferation; genotoxic events induced by tetrachloroethylene metabolites, including chromosomal aberrations; and other nongenotoxic events—such as promotion of growth of previously initiated foci, changes in epigenetic status, and oxidative stress—may all contribute to the overall MOA through several simultaneous mechanisms. The hypothesis that the mutagenic metabolites of tetrachloroethylene (tetrachloroethylene-epoxide, TCA, DCA, chloral hydrate [if it is formed], and TCVG) initiate carcinogenesis and that tetrachloroethylene-induced promotion of initiated foci, cytotoxicity, and epigenetic events promote carcinogenesis cannot be ruled out.

SUMMARY

As with other halogenated solvents, there is evidence in a number of species that tetrachloroethylene can cause liver damage. This was well described by

EPA in the drat IRIS assessment. Two rodent bioassays have demonstrated that high doses of tetrachloroethylene produced liver tumors in mice. While there is clear evidence that this occurs, the basis for their occurrence is not clear and may actually involve more than one MOA. This makes the determination of the relevance to humans more difficult. This is particularly true with respect to the importance of PPARα as the predominant or sole MOA, which led to a split opinion among committee members and a dissenting statement (see Appendix B).

Further studies are needed to define the MOAs for tetrachloroethylene-induced liver tumors, with particular emphasis on the importance of PPARα and whether species difference might exist. In addition, further study is needed to determine the relative roles of metabolites of tetrachloroethylene in tumor development. This may require the development of better analytical methods to detect some metabolites.

7

Renal Toxicity and Cancer

This chapter reviews information presented in the Environmental Protection Agency (EPA) draft Integrated Risk Information System (IRIS) assessment of the toxic and carcinogenic effects of tetrachloroethylene on the kidney. The metabolism of tetrachloroethylene by the kidney is critical for its toxicity and carcinogenicity in that organ. The major metabolites of tetrachloroethylene responsible for renal effects are formed by the glutathione metabolic pathway (see Chapter 2 for an overview of toxicokinetics). The following sections address renal toxicity and carcinogenicity separately, but they are not necessarily independent end points. This information is considered in the context of the other evidence on carcinogenicity in Chapter 11, where EPA's assessment of carcinogenic risks posed by tetrachloroethylene is evaluated.

HUMAN STUDIES

Renal Toxicity

The draft IRIS assessment notes that published information on renal toxicity in humans is not well developed. That is because typical screening tests that use plasma are insensitive. For instance, blood urea nitrogen and creatinine, which accumulate in plasma when glomerular filtration is diminished, do not increase until renal function is about half of normal, and urinalysis is not typically performed. Epidemiologic studies of the effects of tetrachloroethylene exposure on renal function have been reported, and EPA summarizes the findings in a table. The discussion focuses on urinary proteins that are indicative of tubular damage, because β-lyase is found in the proximal tubule. The strengths and weaknesses of the various studies are noted by EPA, and consistencies and inconsistencies are discussed. In general, different reports examined different urinary proteins, which have different sensitivity and selectivity as markers of tubular function. Estimated exposure differed among the reports, as did the number of subjects. Effects on glomerular function, as assessed on the basis of

albuminuria, are discussed briefly. The draft IRIS assessment notes that the results are contradictory. It should also note that some albumin is normally filtered, so small increases in the amount of albumin in the urine can be indicative of tubular damage (the result of failure to reabsorb the small amount filtered). EPA's table should also include the negative findings on albumin in studies by Verplanke et al. (1999) and Lauwerys et al. (1983) and on total protein by Vyskocil et al. (1990). EPA concluded that the epidemiologic studies provided evidence suggestive of subtle damage in renal tubules. The committee agrees with that assessment.

Renal Carcinogenicity

Several types of epidemiologic studies have been used to explore a possible association between jobs in which workers are exposed to tetrachloroethylene and renal-cell carcinoma (RCC), including cohort mortality studies, case-control studies, and nested case-control studies. Ultimately, the methodologic challenges of studying such a rare cancer as RCC, assessing tetrachloroethylene exposure accurately, and evaluating inconsistencies in results among studies limit the conclusions that can be drawn from the epidemiologic data. Most of the studies either did not have explicit information about exposures or had considerable methodologic limitations.

Pesch et al. (2000) conducted a population-based case-control study in Germany that estimated tetrachloroethylene exposure with a job-exposure matrix (JEM) and a job-task exposure matrix (JTEM). The latter is usually superior for estimating specific exposures. The data were acquired in in-person interviews, so information on occupational history was obtained and confounding covariates (such as smoking) were well measured. An increased odds ratio (OR) for tetrachloroethylene exposure was observed in men who had a medium exposure index (OR, 1.4; 95% confidence interval [CI], 1.1-1.7) or a substantial exposure index (OR, 1.4; 95% CI, 1.1-2.0) on the basis of the JEM. However, the results based on the JTEM were less convincing (OR, 1.2; 95% CI, 0.9-1.7 and OR, 1.3; 95% CI, 0.7-2.3 for medium and substantial exposure, respectively). In contrast, no association was observed in women on the basis of the JEM, but a positive albeit imprecise association was observed on the basis of the JTEM for medium and substantial exposure (OR, 2.2; 95% CI, 0.9-5.2 and OR, 2.0; 95% CI, 0.5-7.8, respectively). Those variable results are representative of inconsistencies among studies. Lynge et al. (2006) (listed in Table 4B-4 of the EPA draft but not discussed in the renal-cancer section) conducted a nested case-control study in four Scandinavian countries in a cohort of about 47,000 persons employed in the laundry and dry-cleaning industry as of 1970 and followed through 1997-2001 to identify incident cancers. Multiple cancers were assessed, including 56 RCC cases in men and 154 in women. The cohort was divided into those who were not exposed to the dry-cleaning process, dry-cleaners and other exposed workers, and others working in dry-cleaning. Risk was also estimated by

duration of employment in dry-cleaning occupations. Tetrachloroethylene was the most commonly used solvent in dry-cleaning during the study interval; the mean concentration over the interval of the study was estimated as 24 ppm. The adjusted relative risk of RCC for dry-cleaners compared with unexposed workers was 0.67 (95% CI, 0.43-1.05) on the basis of 29 cases in the exposed. There was no evidence of increasing risk with increasing duration of employment as dry-cleaners. Mandel et al. (1995) pooled data from a multicenter international case-control study of RCC; the study was conducted in six centers in five countries (Australia, Denmark, Germany, Sweden, and the United States) and included 1,732 cases and 2,309 controls. Occupational histories, collected in in-person interviews, were used to estimate exposures to specific chemicals or tasks. The study reported an increased OR of 1.4 (95% CI, 1.1-1.7) associated with exposure to dry-cleaning solvents, but no exposure response was observed on the basis of duration of exposure.

Several other studies, although methodologically sound, were too small or did not have sufficient information about exposure to be informative (Aschengrau et al. 1993; Mellemgaard, et al. 1994; Schlehofer et al. 1995; Dosemeci et al. 1999).

There are inconsistencies in the draft IRIS assessment. Nine studies are listed as larger case-control studies. Of them, EPA judged the case-control studies of Aschengrau et al. (1993), Partanen et al. (1991), and Pesch et al. (2000) to be of high quality, citing exposure information, adequate control of confounding, and histologic confirmation. It is then noted that "these two case-control studies carry greater weight than observations in the other case-control studies identified in Table 4B-4." The Aschengrau et al. study is not listed in Table 4B-4; and this suggests that the Partanen et al. and Pesch et al. studies are those considered to be the studies given greater weight. The point should be clarified. The Lynge et al. (2006) study is not discussed in the "Kidney Cancer in Humans" section of the draft IRIS assessment.

Overall, the epidemiologic literature provides little support for a causal association between tetrachloroethylene exposure and cancer of the kidney. Study results are inconsistent. In addition, those studies that tried to assess dose-response by using the imperfect surrogate of "duration of exposure," found no association between duration and risk. EPA's assessment of the data appropriately labels the evidence supporting an association between tetrachloroethylene and renal cancer as "limited," and the epidemiologic evidence does not appear to weigh heavily toward classifying tetrachloroethylene as a likely carcinogen.

ANIMAL STUDIES

Renal Toxicity

The draft IRIS assessment summarizes the studies of tetrachloroethylene toxicity across species, sexes, and routes and durations of exposure. Significant renal toxicity has been observed in lifetime bioassays in rats and mice of both

sexes (NCI 1977; NTP 1986; JISA 1993). Degenerative changes in the proximal tubule are reported as cloudy swelling, fatty degeneration, and necrosis of the epithelium. Some tubules were filled with hyaline casts; inflammatory cells, fibrosis, and focal mineralization were also reported. Effects of shorter exposures depended on route, duration, and dose. In short term (28-42 days) gavage studies, male rats showed signs of renal damage (Green et al. 1990; Philip et al. 2007). Inhalation exposure of male and female rats and mice to tetrachloroethylene for 28 days caused no effects at 400 ppm, and exposure of male rats for 10 days at 1,000 ppm resulted in an increase in hyaline droplets (Green et al. 1990). Inhalation exposure to tetrachloroethylene for 13 weeks resulted in karyomegaly in male and female mice but not in rats (NTP 1986); the response was minimal at 200 ppm and increased in severity with exposure concentration.

Renal Carcinogenicity

Renal-tubular adenoma and carcinoma were observed in male rats in the NTP (1986) bioassay and to a lesser extent in the Japan Industrial Safety Association (JISA 1993) studies. Tetrachloroethylene caused a low rate of induction of renal tumors in rats; although the yield at the high dose was not statistically significant. In the NTP bioassay, induction of renal tumors was dose-dependent. The incidence was 1 of 49 in the control group, 3 of 49 in the 200-ppm group, and 4 of 50 in the 400-ppm group. There are wide confidence limits on the data, and some of the error bars approach zero. There is a very low spontaneous incidence of renal tumors in Fischer 344 rats (Haseman et al. 1998). Induction of renal tumors in rats by tetrachloroethylene is therefore easily observed against a low background. In addition, the controls had only benign tumors, not malignant tumors, whereas the high-dose group had two malignant tumors. In the draft IRIS assessment, EPA calculates the chance that two animals will have a rare tumor to be less than 0.001, giving biological relevance to the finding. Maltoni and Cotti (1986) observed no increase in kidney tumors following tetrachloroethylene administration by gavage to male Sprague-Dawley rats. Overall, the dose-dependent induction of renal tumors in one experiment against the low background incidence of renal tumors in rats exposed to tetrachloroethylene indicates that tetrachloroethylene can induce renal tumors in rats. After integrating the results of the studies, the committee concluded that tetrachloroethylene induces renal tumors in rats. EPA considers the renal tumors to be suggestive of an effect and notes that it is similar to the effects of other chlorinated ethanes and ethylenes. The committee agrees with EPA's assessment.

Mode of Action

EPA considered key events and potential modes of action for renal-tumor formation following tetrachloroethylene exposure and concluded that the mechanisms are not understood.

The draft IRIS assessment discusses an $\alpha_{2\mu}$-globulin nephropathy mode of action of tetrachloroethylene-induced renal carcinogenesis in detail. Renal tumors that arise solely by $\alpha_{2\mu}$-globulin nephropathy are not considered relevant to human risk assessment, because $\alpha_{2\mu}$-globulin nephropathy is specific to the male rat. Although hyaline droplets that contain $\alpha_{2\mu}$-globulin have been reported after exposure to high concentrations of tetrachloroethylene, the histopathologic findings reported in the inhalation bioassays were not consistent with the $\alpha_{2\mu}$-globulin-mediated mode of action (NTP 1986; JISA 1993). Gavage bioassay (NCI 1977) showed that histopathologic characteristics were more consistent with $\alpha_{2\mu}$-globulin nephropathy. However, in all these bioassays, similar histopathologic findings in the kidney were reported in female rats and male and female mice. These positive responses are not consistent with the male rat specificity of the $\alpha_{2\mu}$-globulin nephropathy mode of action and therefore contradict a role of $\alpha_{2\mu}$-globulin nephropathy in renal tetrachloroethylene tumorigenesis. The committee agrees with EPA's assessment that $\alpha_{2\mu}$-globulin nephropathy is not supported as a mode of action in tetrachloroethylene-induced renal carcinogenesis.

Tetrachloroethylene can stimulate the peroxisome proliferation response, as indicated by cyanide-insensitive palmitoyl CoA oxidation activity, in the kidneys of mice but not rats (Goldsworthy and Popp 1987). Odum et al. (1988) reported similar findings; mouse kidney samples were pooled for assays, so statistical analysis was not conducted on mouse kidneys. The peroxisome proliferation response does not correlate with tumor response and therefore is not consistent with a role of peroxisome proliferation as a mode of action in renal tumorigenesis. EPA notes that activation of peroxisome proliferator-activated receptors has not been established as a mode of renal tumorigenesis. The committee agrees that the data do not support peroxisome proliferation as a mode of action.

The draft IRIS assessment also considers immunotoxicity and immunosuppression as a mode of action of tetrachloroethylene tumorigenesis. In humans, immune-mediated renal damage is most often seen as damage to the glomeruli. The reports of renal damage in humans are based on abnormal protein in the urine; the pattern of proteinuria is indicative of tubular, not glomerular, damage. Thus, the type of renal damage seen is not consistent with an immunotoxic mode of action. The draft IRIS assessment notes that immune-system-mediated effects of organic solvents and the formation of protein adducts are related to autoimmune diseases, not to immunosuppression and therefore inconsistent with immunosuppression as a mode of action.

Tetrachloroethylene causes toxic nephropathy in high doses, and this was observed in the cancer bioassay studies (NCI 1977; NTP 1986; JISA 1993). EPA considered a mode of action in which renal cytotoxicity and subsequent proliferation—as part of the repair process, not associated with $\alpha_{2\mu}$-globulin—result in renal-tubular neoplasia. Renal toxicity has been observed with various metabolites of tetrachloroethylene (Lash et al. 2007; Elfarra and Krause 2007). Each of the three major metabolic pathways of tetrachloroethylene yields metabolites that are cytotoxic (Dekant et al. 1986c, 1988; Vamvakas et al. 1989a,c;

DeMarini et al. 1994; Werner et al. 1996; Volkel and Dekant 1998; Muller et al. 1998a; Dreessen et al. 2003). Chronic nephrotoxicity has been reported in male rats at the termination of all long-term bioassays but also has been observed in chronic bioassays at 2 years in female rats and both sexes of mice, none of which develop tumors. Despite this inconsistency, it is not possible to rule out a role of chronic toxicity in tumor formation.

The draft IRIS assessment concludes that a mutagenic mode of action cannot be ruled out. The committee agrees with this assessment. A mutagenic mode of action is supported by the findings after exposure to the structurally similar trichloroethylene. Some metabolites derived from *S*-(1,2,2-trichlorovinyl) glutathione (TCVG), the glutathione conjugate of tetrachloroethylene, have been shown to be mutagenic in bacterial systems (Vamvakas et al. 1989a,d) or to cause unscheduled DNA synthesis (Vamvakas et al. 1989c). Others react with DNA in vitro (Muller et al. 1998a,b). *S*-(1,2,2-Trichlorovinyl)-L-cysteine (TCVC) causes a greater response than dichlorovinyl cysteine in mutagenicity tests using *Salmonella* (Dekant et al. 1986c) and in renal toxicity (Birner et al. 1997). Tetrachloroethylene has not been shown to be mutagenic with or without activation by S9 in *Salmonella* or in mammalian cells. However, when tetrachloroethylene was activated with purified glutathione *S*-transferase, glutathione, and rat kidney fractions, TCVG was formed, and consequent mutagenic activity in *Salmonella* was clearly demonstrated, as described by EPA. S9 activation of tetrachloroethylene did not induce mutation in cultured mouse lymphoma L5178Y cells.

SUMMARY AND RECOMMENDATIONS

EPA concluded there is limited evidence that tetrachloroethylene causes cancer in humans, and the committee agrees with this assessment. EPA evaluated bioassay studies to provide evidence suggestive of an effect. The committee considers this and the similarity to trichloroethylene to support the conclusion that tetrachloroethylene induces kidney tumors in rodents. While the mode of action of tetrachloroethylene tumorigenesis is not understood, the $\alpha_{2\mu}$-globulin nephropathy and peroxisome proliferator modes of action are not consistent with experimental results. A mutagenic mode of action cannot be ruled out.

Further studies are needed to determine whether tetrachloroethylene and its metabolites formed from TCVG (TCVC, chlorothioketene, and sulfoxide metabolites) are mutagenic in other mammalian cell assays (mutation to 6-thioguanine resistance in cultured V79 Chinese hamster lung fibroblasts or in Chinese hamster ovary cells). It is possible that any of the metabolites of TCVG contribute to the carcinogenicity of tetrachloroethylene in rat kidney, but this needs to be studied. Further data on the sequencing of DNA from tetrachloroethylene-induced renal tumors for mutations of the von Hippel Landau tumor-suppressor gene, other tumor-suppressor genes and oncogenes, and their downstream effectors (for example, p27 that controls cell-cycle progression) are needed to determine whether TCVG and similar tetrachloroethylene metabolites

are causing or contributing to the formation of renal tumors. Finally, a robust physiologically based pharmacokinetic model is needed to evaluate differences between humans and rats in their sensitivity to tetrachloroethylene.

8

Hematopoietic Effects

This chapter reviews information presented in the Environmental Protection Agency (EPA) draft Integrated Risk Information System (IRIS) assessment of the effects of tetrachloroethylene on the hematopoietic system, especially the development of mononuclear-cell leukemia (MCL) in rats and lymphomas in humans. The information is considered in the context of the other evidence on carcinogenicity in Chapter 11, where EPA's assessment of carcinogenic risks of tetrachloroethylene is evaluated.

ANIMAL STUDIES

The draft IRIS assessment proposes to use the finding of MCL in male F344 rats as the most sensitive tumor response, supporting its weight-of-evidence classification of tetrachloroethylene as "likely to be carcinogenic to humans" by all routes of exposure. The use of MCL to support that conclusion is based primarily on two studies: those of the National Toxicology Program (NTP 1986) and the Japan Industrial Safety Association (JISA 1993). Both studies reported that chronic inhalation administration of tetrachloroethylene to male and female F344 rats caused "positive trends" in MCL with increasing dose. As the draft IRIS document correctly points out, the scientific reliability of those studies has been questioned in part because of "high spontaneous background incidences, use of special supplemental analysis to aid in data interpretation, and the relevance of MCL in F344/N rats to human hazard" (p 4-159, lines 21-23). The committee similarly questions the use of the tetrachloroethylene exposure bioassays in the F344 rat for cancer risk assessment for those reasons and others discussed below.

In the NTP (1986) study, F344/N male and female rats were exposed chronically to tetrachloroethylene at 200 and 400 ppm. The incidence of MCL in males was 77% in the 200-ppm group and 74% in the 400-ppm group, and in females 60% and 58%, respectively. The background incidences of MCL in the controls were high (56% in males and 36% in females). Such high backgrounds

make it difficult to interpret the biological significance of the increase in the incidence of MCL observed in the treatment groups. Indeed, NTP has decided to stop using its F344/N rat colony in its bioassays for reasons that include the high background rate of MCL (King-Herbert and Thayer 2006). A supplemental analysis performed by NTP considered disease progression, latency, and various statistical treatments. The analysis suggested an increase in tumor incidence over controls at both test concentrations despite the high spontaneous tumor incidence in the controls.

The significance of MCL findings in multiple NTP bioassays that used the F344 rat was the subject of a recent reanalysis by Thomas et al. (2007), which EPA should reference in the draft IRIS assessment. They examined the incidence of leukemia in 2-year bioassays that included untreated male and female F344 rats from 1971 to 1998. They found that background tumor incidence increased substantially, from 7.9% to 52.5% in males and from 2.1% to 24.2% in females, over that period. The analysis also found that MCL responses are highly variable and subject to substantial modulation by dietary factors.

Thomas et al. (2007) also evaluated MCL incidence in male and female rats exposed to 500 chemicals. On the basis of 34 NTP studies that yielded evidence of a chemically related increase in the incidence of leukemia, which included the 1986 NTP study of tetrachloroethylene, the authors conducted a reanalysis of dose-response data by comparing results with four statistical methods: Fisher's test for pair-wise comparison of leukemia incidence between a dose group and a control group, the Cochran-Armitage test for incidence trend, logistic regression for incidence, and life tables for survival-adjusted incidence. Tetrachloroethylene was one of five chemicals shown by the authors to produce leukemia in both sexes of rats. They used the rigid Food and Drug Administration (FDA) statistical criteria for testing dose-related cancer incidences ($p < 0.01$ for pairwise comparison; $p < 0.005$ for trend test). The results in male rats in the 1986 NTP study revealed a significant dose-response trend when analyzed with a life table ($p = 0.004$) assuming that MCL is lethal but a nonsignificant trend with logistic regression ($p = 0.097$) assuming the MCL is nonlethal. Pairwise comparisons revealed dose-related incidences ($p = 0.046$) for both dose groups, and the trend test yielded a p value of 0.034; neither met the FDA criteria for statistical significance. The borderline significance of the trend test and nonsignificance of logistic regression for the latter two comparisons could be explained in part by the fact that the incidences did not follow an incrementally increasing relationship with dose. In female rats in the NTP study, use of a life table ($p = 0.053$), logistic regression ($p = 0.012$), a trend test ($p = 0.018$), and Fisher's test ($p = 0.014$ and 0.022, respectively, for two doses) all revealed a borderline significant dose-related incidence. However, there is inconsistency in statistical significance between the sexes and uncertainty about the shape of the dose-response curve, especially in the lower range of the study. The authors recommended the use of life-table analysis for survival-adjusted leukemia incidence, noting that it is "closer to reflecting the true statistical significance of the carcinogenic effect" than logistic-regression treating dose as linear. Life-table

analysis (log-rank test) accounts for time-to-event information, is capable of testing nonlinear dose-response relationships of arbitrary shapes, and is therefore more flexible than the Cochran-Armitage trend test. Survival analysis also is more relevant than logistic regression for more lethal tumors such as MCL. Overall, Thomas et al. showed a moderately significant dose-response relationship for tetrachloroethylene, but this finding should be evaluated by EPA with a weight-of-evidence approach suggested in its 2005 *Guidelines for Carcinogen Risk* before conclusions are drawn.

In the 1993 JISA study, F344/DuCrj rats were exposed to tetrachloroethylene at 50, 200, and 600 ppm. The draft IRIS document focuses on the JISA report for cancer dose-response assessment because the study included a 50-ppm exposure concentration, which is one-fourth the lowest exposure concentration in the 1986 NTP study. As in the NTP study, there was a high incidence of MCL in the controls (22% in males and 20% in females). Against that high spontaneous incidence of MCL, the incidence of MCL in male and female rats exposed to tetrachloroethylene at 50, 200, and 600 ppm was 28%, 44%, and 54% and 34%, 32%, and 38%, respectively. Moreover, the historical rate of MCL for the Japanese laboratory is very high. There was no incremental increase in MCL incidence in female rats with increasing dose. In contrast, EPA concluded that male rats displayed a dose-dependent increase in MCL although in the analysis background values were subtracted from the incidences in animals treated with tetrachloroethylene (Figure 5-6 in the draft IRIS assessment), and this may lead to a false impression. Such manipulation of data is not widely accepted in statistical practice, because it artificially reduces the uncertainty caused by the variation in the background rate. As noted in reviews by Caldwell (1999) and Ishmael and Dugard (2006), the unusually high background rate of MCL in control (untreated) rats weakens the ability to separate the background response from possible chemically induced responses, particularly when the chemically induced response above background is low. The committee recommends that the statistical approaches applied by Thomas et al. (2007) to the NTP study be applied also to the JISA study.

It is unclear whether MCL is a relevant predictor of human leukemias or other adverse health effects. Thomas et al. (2007) argue that MCL is a large granular lymphocytic leukemia (LGLL) of natural-killer (NK) cell origin that shares "some characteristics" with a rare human NK-LGLL. However, they also note that in contrast with F344 rats, human NK-LGL leukemia is rare, occurs primarily in the young, and may be associated with Epstein Barr virus (EBV) although no such virus-leukemia association is known to contribute to the etiology of rat LGLL/MCL. EPA contends that MCL is "similar" to human lymphoid cancers (T-cell and NK-LGL leukemias) and relies on a study (Stromberg 1985) that compared morphologic characteristics between rat MCL and human T-cell lymphoma. EPA considers that to be supportive evidence, despite the fact that these cancers arise in different tissues and that the cell origin in both cases is unknown. EPA states (EPA 2008, p. 4-161) that "discounting a rodent neoplasm simply because it has no human counterpart is not a scientifically defensi-

ble position. Strict site concordance is not a requirement for relevance in extrapolation of hazard potential." The committee agrees with those statements, but notes that the available data should be used to provide a more convincing argument. Similarly, EPA argues that humans are heterogeneous and so could have the same inherited susceptibility as F344 rats, but provides no scientific basis for that argument.

HUMAN STUDIES

Few human data are available for assessing the relationship between tetrachloroethylene exposure and the risk of specific cell types of lymphohematopoietic cancers. Several studies have assessed the risk of chronic lymphocytic leukemia in humans (Morton and Marjanovic 1984; Travier et al. 2002; Ji and Hemminki 2005, 2006), but otherwise the finest classification of outcomes used was "leukemia," "lymphoma," "non-Hodgkin lymphoma" (NHL), and "Hodgkin disease" (HD). The EPA draft IRIS assessment concludes (p. 4-184) that the epidemiologic data "suggested an association between lymphoma and tetrachloroethylene." The committee concurs with that conclusion but would add that the data are relatively weak and inconsistent. Associations between those cancers and exposure to tetrachloroethylene are based on very small numbers and thus are statistically unstable. The positive associations with tetrachloroethylene are sometimes observed only for lymphomas in women: NHL reported by Spirtas et al. (1991) and Anttila et al. (1995) and HD reported by Blair et al. (2003) and Miligi et al. (2006). It is not clear why those differences in sex-specific results appear; they may be due to residual confounding, in that it is unlikely that men would have appreciably lower exposures than women in the same jobs. It is also possible that sex-specific susceptibility issues are contributing to this observation. Other large cohort studies (Boice et al. 1999; Lynge et al. 2006) found no association in either women or men, and no dose-response effects have been observed. Epidemiologic studies of the association vary with study design, validity, specificity of exposure assessment, type of population studied, and sample size, all of which contribute to the inconsistency of results and reduce the committee's confidence in the conclusions that can be drawn from the data. The committee also noted a number of factual errors in this section of the IRIS draft that should be corrected; such errors detract from overall confidence in the draft's conclusions.

MODE OF ACTION

Given the high background rate of MCL in F344 rats, it is important to question whether tetrachloroethylene induces MCL or promotes an increase over the background rate. However, few data are available for addressing the question. According to EPA, a link to a mode of action (MOA) for tetrachloroethylene-induced MCL implicates a circulating genotoxic metabolite that is formed in

the kidney by cleavage of a cysteine conjugate, *S*-(1,2,2-trichlorovinyl)-L-cysteine (TCVC) and may cause DNA damage in bone marrow. The EPA draft discusses studies that showed that a related (trichloroethylene-derived) cysteine conjugate, *S*-(1,2-dichlorovinyl)-L-cysteine, caused DNA alterations and toxicity in the bone marrow, lymph nodes, and thymus of calves (Bhattacharya and Schultze 1971, 1972; Lock et al. 1996). The finding that TCVC did not induce those responses in the same study does not appear to have factored into EPA's support of the hypothesis of a genotoxic MOA. The committee judges that a genotoxic MOA of tetrachloroethylene induction of MCL involving the cysteine conjugate β-lyase pathway is highly speculative and not supported by data.

The committee found some additional data on tetrachloroethylene that might be relevant for MOA analyses. They include studies by Marth et al. (Marth et al. 1985, 1989; Marth, 1987) and a study by Seidel et al. (1992) on tetrachloroethylene toxicity in mice. In the Marth et al. studies, NMRI mice were orally exposed to tetrachloroethylene at 0.05 or 0.1 mg/kg per day for 7 weeks. The mice exhibited a reversible hemolytic anemia and had microscopic evidence of splenic involvement (Marth et al. 1985), and tetrachloroethylene was found to accumulate in the spleen (as shown in Figure 2 of Marth et al. 1989), where MCL is thought to originate. Nevertheless, hemolytic anemia arises as a result of a defect in the mature red-cell membrane, as opposed to the various forms of leukemia which are thought to arise as a result of mutational changes early in bone-marrow-cell differentiation. Thus, hemolysis would not be expected to play a role in leukemogenesis. The observations reported by Marth et al. have not been reproduced or reported by any other laboratory.

Seidel et al. (1992) exposed hybrid mice (C57/BL/6 × DBA/2) to tetrachloroethylene at 270 ppm (11.5 weeks) and 135 ppm (7.5 weeks) 6 hours/day 5 days/week. Reductions in the numbers of lymphocytes/monocytes and neutrophils were observed, but they returned to control values over the next 3 weeks. There were no effects on spleen colony-forming units (CFU-Ss), but evidence of a reduction in red cells was supported by decreases in erythroid colony-forming units and erythroid burst-forming units and evidence of reticulocytosis. The data suggest a reversible bone marrow depression.

Inhibited production of both red cells and various forms of white cells have been reported after exposure to a variety of leukemogens (such as anticancer alkylating agents or benzene). The leukemogens usually decrease CFU-Ss, an effect not observed with tetrachloroethylene exposure (Seidel et al. 1992). They also usually decrease the bone marrow myeloid progenitors, CFU-GEMM, CFU-GM, and CFU-E/BFU-E, the latter of which was also decreased by tetrachloroethylene (Seidel et al. 1992). EPA should consider reviewing the evidence from models of leukemia induced in humans by chemicals (such as benzene and chemotherapeutic agents) to determine whether there are similarities with tetrachloroethylene-induced MCL.

The Marth et al. studies and the Seidel et al. study provide indirect evidence that tetrachloroethylene exposure induces effects associated with MCL and known leukemogens, respectively, but are insufficient to support the argu-

ment that tetrachloroethylene induces MCL or a related form of leukemia. In addition, those studies investigated tetrachloroethylene exposure in mice, a species in which MCL has never been observed. The only evidence that tetrachloroethylene induced MCL comes from exposure studies with F344 rats. Nevertheless, the effects of tetrachloroethylene on hemolysis in mice and on bone marrow function provide the basis of a hypothesis that could be explored to demonstrate the mechanism by which tetrachloroethylene could, within some dose range, affect the spleen.

SUMMARY

The majority of the committee finds that EPA has not adequately justified the use of MCL data over the evidence for liver or kidney cancer in its cancer risk assessment. Evidence of tetrachloroethylene-induced leukemia from epidemiologic studies is limited and inconsistent. The NTP (1986) and JISA (1993) study results of increased MCL incidences in F344 rats given tetrachloroethylene by inhalation are also questionable because of the high background rates of MCL in control animals. More thorough statistical evaluation of the data, such as the life-table analysis proposed by Thomas et al. (2007), could provide a stronger basis for drawing conclusions. However, MCL resulting from tetrachloroethylene exposure has not been observed in other strains of rats or other animal species, and no definitive evidence is available to support a hypothesized MOA by which tetrachloroethylene increases MCL in F344 rats. Those are all sources of uncertainty surrounding the relevance of MCL to human cancer risk. The information is considered in the context of the other evidence on carcinogenicity in Chapter 11, where EPA's assessment of carcinogenic risks of tetrachloroethylene is evaluated.

9

General Review of Epidemiologic Evidence Pertaining to Cancer

The Environmental Protection Agency (EPA) draft Integrated Risk Information System (IRIS) assessment of tetrachloroethylene characterizes the epidemiologic literature as supportive of classifying tetrachloroethylene as a likely carcinogen. That classification is based primarily on reported associations with hematopoietic, lymphopoietic, and esophageal cancers. There is a substantial epidemiologic literature on the potential association of exposure to tetrachloroethylene with selected malignancies. However, the committee believes that a balanced and critical review of the human epidemiologic literature provides only limited evidence that tetrachloroethylene is carcinogenic in humans. The challenges of obtaining valid estimates of exposure, in addition to the challenges inherent in observational epidemiology, make it difficult to draw conclusions about causal associations between tetrachloroethylene and cancer in humans.

The epidemiologic literature relating tetrachloroethylene to cancer is notable in three ways: a number of studies show associations with a variety of cancers, there is limited consistency between studies with respect to the associations, and few studies were able to quantify or even identify specific tetrachloroethylene exposure. The latter point, not uncommon in studies of occupational and environmental causes of cancer, makes interpretation of the literature particularly difficult. Several positive associations are reported in the literature, but the inconsistency among studies raises concern, so a consistent critical review of the literature is needed. The draft IRIS assessment does not provide the detail and methodology used for evaluating literature. Overall, it appears that the procedure was to accept the results of positive studies with little critical evaluation of validity and to dismiss null studies of similar or better methodologic rigor as flawed. If it is EPA's intention to err on the side of protecting public health when reviewing the literature, that should be stated clearly in the document. Otherwise, a clearer discussion of criteria used to identify studies of merit and a more balanced critique would strengthen the draft IRIS assessment.

The draft's critiques of studies are often uneven; studies that found no association are criticized more often than studies that found a positive association even if they had similar methodologic limitations. An example is the discussion of case-control studies on page 4-150, lines 19-31. Several of the criticized features of the case-control design that are mentioned are not inherent in the design, such as that associations may be nonlinear (this design does not require categorical exposure measures) or that duration and cumulative exposure do not address age at first exposure (this information can simply be asked of participants). Many of the studies suffered from a lack of statistical power—a common problem in studying rare cancers and exposures. However, the concern over power is uneven. On page 4-149, the absence of an association between employment in dry-cleaning and death due to lymphatic and hematopoietic cancer (Ruder et al. 2001) is attributed to lack of power. In contrast, a positive association between exposure to tetrachloroethylene and multiple myeloma in aircraft maintenance workers was based on only two deaths and is described only as noteworthy but imprecise (page 4-148, lines 6-9). There is little discussion of the potentially important limitations of proportionate-mortality studies, such as inaccuracies in death certification and the inability to adjust for potential confounders. There is some discussion of confounders in relation to the standardized mortality ratio (SMR) studies of esophageal cancer on page 4-153, but it is also unbalanced in that it focuses on adjustment for smoking but does not mention the absence of adjustment for alcohol; in addition, the effect of adjustment for smoking is derived from estimates for lung cancer and may not translate directly to esophageal cancer.

A number of errors suggest an incomplete understanding of epidemiologic and statistical methods. Such errors reduce confidence in the draft's conclusions. For example, EPA summed observed and expected cases from studies with diverse types of end points (incidence and mortality) and, using different approaches to calculating the expected values, calculated a ratio of the summed observed and expected values. Expected numbers from different studies can be added only if they are derived from the same external rates, but mortality and incidence are different. One of the most troubling misunderstandings is related to the dismissal of the results of the 2006 study by Lynge et al. In reference to that study's findings on non-Hodgkin lymphoma (and later on bladder cancer), EPA notes that exposure information was not available on about 20% of cases and of controls and that much of the exposure information came from next of kin. It then uses that to explain why Lynge et al. found no risk associated with tetrachloroethylene exposure and suggests an automatic bias toward the null due to misclassification. In the first instance, missing exposure data are analogous to nonresponse in that the subjects are not included in any classification group. Nonresponse will not introduce bias if it is nondifferential; if it is differential, it could bias an effect measure either toward or away from the null. In the second instance, exposure information from next of kin make it more likely that hazardous exposures will be overreported by the families of workers who developed cancer than by families of workers who did not; this would have resulted in

overestimation, not attenuation, of the association. Similar arguments regarding the study are incorrectly made for other cancer sites, and the draft refers to the study as "uninformative." It is unclear why Lynge et al. (2006) received such critical review and papers that were methodologically less sound were accepted with little comment.

The draft IRIS assessment indicated that the strongest evidence linking tetrachloroethylene to cancer consisted of observed associations with esophageal cancer and lymphoma (page 4-184, lines 6-17). Evidence on other cancer end points—including renal, bladder, cervical, and lung cancers—is less certain and does not weigh as heavily in the assessment (page 4-184, lines 25-33). After a brief and uncritical discussion of the epidemiologic literature that references the criteria for causation outlined by Hill (1965), the document concludes that "together, the evidence on tetrachloroethylene partially fulfills several of these criteria and is suggestive of a cause and effect relationship between tetrachloroethylene and human cancer. The body of human evidence *is not sufficient* to regard tetrachloroethylene as a known human carcinogen" (p. 44-187; emphasis added). In contrast, in Chapter 6 of the draft ("Characterization of Hazard and Dose-Response"), the evidence associating tetrachloroethylene exposure with cancer is stated more confidently (page 6-5, lines 31-35; page 6-6, lines 1-5; page 6-10, lines 27-29 and 31-35; and page 6-11, lines 1-6). It is difficult to reconcile the discussion in Chapter 4 with the conclusion in Chapter 6.

ESOPHAGEAL CANCER

The draft IRIS assessment emphasizes the association between tetrachloroethylene and esophageal cancer primarily because of the results of three studies: by Vaughan et al. (1997), Ruder et al. (2001), and Blair et al. (2003). The work by Blair et al. and Ruder et al. were mortality studies of dry-cleaner union members, and the latter was a community-based case-control study. It is interesting to compare the results of the two studies. With the same methods, the populations were enumerated from similar sources and followed for similar periods. Blair et al. followed 5,369 union members in St. Louis who worked for at least 1 year during 1948-1993. The population studied by Ruder et al. included 1,708 workers selected from union rosters in California, Illinois, Michigan, and New York. Both studies reported an excess risk of death from esophageal cancer; Blair et al. reported an SMR of 2.2 (95% confidence interval [CI], 1.5-3.3) and Ruder et al. an SMR of 2.47 (95% CI, 1.35-4.14). The excess in the paper by Ruder et al. was limited to workers with at least 20 years since first employment and was highest in those with at least 5 years of exposure (SMR, 5.03; 95% CI, 2.41-9.47). Blair et al. reported similar SMRs in workers with little or no exposure (SMR, 2.1; 95% CI, 0.9-4.4) and those with medium or high exposure (SMR, 2.2; 95% CI, 1.1-3.5).

Esophageal cancer is also associated with smoking and alcohol consumption, which are difficult to control for in mortality studies because the data are

often not available. The studies of Blair et al. and Ruder et al. also reported an excess of deaths from other causes associated with smoking, including lung cancer, emphysema, and heart disease. EPA's draft IRIS assessment discounts potential confounding by smoking but does not adequately support its conclusion in the section on esophageal cancer (page 4-153, lines 30-33). In contrast with the findings of Blair et al. and Ruder et al., a large mortality study (Boice et al. 1999) in a population of aircraft manufacturers (N = 77,965) had an appreciable number of workers with routine (N = 2,631) and intermittent (N = 3,199) exposure to tetrachloroethylene but reported no association between that exposure and esophageal cancer. The case-control study by Vaughan et al. (1997) reported an increased but not significant odds ratio (OR) for dry-cleaning work, which was adjusted for smoking habit and alcohol consumption. That estimate was based on only two exposed cases, however, and, particularly when multiple covariates were adjusted for, was too statistically unstable to be informative (OR 3.6; 95% CI, 0.5-27.0). A methodologically sound nested case-control study by Lynge et al. (2006) reported no association between working as a dry-cleaner and esophageal cancer. Those negative findings were dismissed by EPA because some of the population could not be classified by exposure. As discussed earlier, this does not preclude the use of results based on subjects on whom exposure data were available.

Overall, there is limited evidence to support an association between tetrachloroethylene and esophageal cancer. The two mortality studies of dry-cleaners are suggestive of an association, but the potential for confounding by smoking and alcohol consumption is appreciable. Thus, the committee therefore concluded that the epidemiologic literature is not sufficient to support an association between tetrachloroethylene and esophageal cancer.

LYMPHOID CANCERS

EPA's draft IRIS assessment concludes that the epidemiologic data "suggested an association between lymphoma and tetrachloroethylene" (p. 4-184). The committee concurs with that conclusion but adds that the data are relatively weak and inconsistent. The rationale for the committee's conclusion is discussed in detail in Chapter 8.

Epidemiologic studies of the association between exposure to tetrachloroethylene and lymphoid cancers vary in design, validity, specificity of exposure assessment, type of population studied, outcome, and sample size, all of which contribute to the inconsistency of results and reduce confidence in conclusions that are drawn from the data.

OTHER CANCERS

A number of studies have reported associations between tetrachloroethylene and other cancers, including cervical, lung, and bladder cancer. The results